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NOVEL PROTEINS AND THEIR USES

FIELD OF THE INVENTION

5 The present invention relates to the identification and isolation of novel DNA, therapeutic and drug discovery uses, and the recombinant production of novel secreted polypeptides, designated herein as LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419. The present invention also relates to vectors, host cells, and antibodies directed to these polypeptides.

BACKGROUND OF THE INVENTION

10 The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by
15 secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

20 Many secretory proteins, including cytokines, have proven to be therapeutically beneficial. Cytokines are diverse proteins that elicit a variety of cellular responses through cellular signaling that occurs during immune responses. The signal transduction

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process through which cytokines elicit particular responses from cells involves a series of protein-protein recognition events, including the binding of a given cytokine to a specific, transmembrane receptor on a cell surface. In addition to cytokines themselves, cytokine receptors also have potential as therapeutic or diagnostic agents.

5 Cytokines identified to date include interleukin-25 (IL-25, which has also been referred to as IL-17E), a pro-inflammatory cytokine which is structurally related to IL-17 [Fort *et al.*, *Cell* 15:985-995 (2001); Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)]. The family of IL-17 cytokines have been implicated in several diseases and conditions. The receptor for IL-25 (IL-25R, also referred to as IL-17Rh1, Evi27 protein, or IL-17BR)
10 has also been reported [WO 00/55204; WO 01/68705; Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001); Tian *et al.*, *Oncogene* 19:2098-2109 (2000); Shi *et al.*, *J. Biol. Chem.* 275:19167-19176 (2001)].

The discovery of novel proteins derived from cytokine receptors and the polynucleotides that encode them satisfies a need in the art by providing new
15 compositions that are useful in the diagnosis, prevention, and/or treatment of medical diseases, disorders, and/or conditions, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of proteins.

The present invention describes the cloning and characterization of novel proteins, termed LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399,
20 LP417, LP418, LP419 as well as active variants and/or fragments thereof.

SUMMARY OF THE INVENTION

The present invention provides isolated LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, and LP419 polypeptide encoding nucleic
25 acids and the polypeptides encoded thereby, including fragments and/or specified variants thereof. Contemplated by the present invention are LP probes, primers, recombinant vectors, host cells, transgenic animals, chimeric antibodies and constructs, LP polypeptide antibodies, as well as methods of making and using them diagnostically and therapeutically as described and enabled herein.

30 The present invention includes isolated nucleic acid molecules comprising polynucleotides that encode LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, and LP419 polypeptides as defined herein, as well as

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fragments and/or specified variants thereof, or isolated nucleic acid molecules that are complementary to polynucleotides that encode such LP polypeptides, or fragments and/or specified variants thereof as defined herein.

A polypeptide of the present invention includes an isolated LP polypeptide
5 comprising at least one fragment, domain, or specified variant of at least 90-100% of the contiguous amino acids of at least one portion of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

The present invention also provides an isolated LP polypeptide as described herein, wherein the polypeptide further comprises at least one specified substitution,
10 insertion, or deletion corresponding to portions or specific residues of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

The present invention also provides an isolated nucleic acid probe, primer, or fragment, as described herein, wherein the nucleic acid comprises a polynucleotide of at least 10 nucleotides, corresponding or complementary to at least 10 nucleotides of SEQ
15 ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23.

The present invention also provides compositions, including pharmaceutical compositions, comprising an LP polypeptide, an LP polypeptide-encoding polynucleotide, an LP polynucleotide, and/or an LP polypeptide antibody, wherein the composition has a measurable effect on an activity associated with a particular LP
20 polypeptide as disclosed herein. A method of treatment or prophylaxis based on an LP polypeptide associated activity as disclosed herein can be effected by administration of one or more of the polypeptides, nucleic acids, antibodies, vectors, host cells, transgenic cells, and/or compositions described herein to a mammal in need of such treatment or prophylactic. Accordingly, the present invention also includes methods for the
25 prophylaxis or treatment of a patho-physiological condition in which at least one cell type involved in said condition is sensitive or responsive to an LP polypeptide, LP polypeptide-encoding polynucleotide, LP nucleic acid, LP polypeptide antibody, host cell, transgenic cell, and/or composition of the present invention.

The present invention also provides an article of manufacture comprising a
30 container, holding a composition effective for treating a condition disclosed herein, and a label.

The present invention also provides a method for identifying compounds that bind an LP polypeptide, comprising:

a) admixing at least one isolated LP polypeptide as described herein with a test compound or composition; and

5 b) detecting at least one binding interaction between the polypeptide and the compound or composition, optionally further comprising detecting a change in biological activity, such as a reduction or increase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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The polynucleotides and polypeptides of the present invention are designated herein as "LP polynucleotides" or "LP polypeptide-encoding polynucleotides" and "LP polypeptides." When immediately followed by a numerical designation (i.e., LP391), the term "LP" refers to a specific group of molecules as defined herein. A complete
15 designation wherein the term "LP" is immediately followed by a numerical designation and a molecule type (i.e., LP391 polypeptide) refers to a specific type of molecule within the designated group of molecules as designated herein.

The terms "LP polypeptide-encoding polynucleotides" or "LP polynucleotides" and "LP polypeptides" wherein the term "LP" is followed by an actual numerical
20 designation as used herein encompass novel polynucleotides and polypeptides, respectively, which are further defined herein. The LP molecules described herein may be isolated from a variety of sources including, but not limited to human tissue types, or prepared by recombinant or synthetic methods.

One aspect of the present invention provides an isolated nucleic acid molecule
25 comprising a polynucleotide which encodes an LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419 polypeptide as defined herein. In a preferred embodiment of the present invention, the isolated nucleic acid comprises 1) a polynucleotide encoding an LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419 polypeptide having an amino acid sequence as
30 shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 respectively; 2) a polynucleotide complementary to such encoding nucleic acid sequences, and which

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remain stably bound to them under at least moderate, and optionally, high stringency conditions; or 3) any fragment and/or variant of 1) or 2).

The term "LP polypeptide" specifically encompasses truncated or secreted forms of an LP polypeptide, (e.g., soluble forms containing, for instance, an extracellular
5 domain sequence), variant forms (e.g., alternatively spliced forms) and allelic variants of an LP polypeptide.

In one embodiment of the invention, the native sequence LP polypeptide is a full-length or mature LP polypeptide comprising amino acids as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24. Also, while the LP polypeptides disclosed herein
10 are shown to begin with a methionine residue designated as amino acid position 1 it is conceivable and possible that another methionine residue located either upstream or downstream from amino acid position 1 may be employed as the starting amino acid residue.

A "portion" of an LP polypeptide sequence is at least about 20, 25, 30, 35, 40, 45,
15 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 contiguous amino acid residues in length. "LP polypeptide variant" is intended to refer to an "active" LP polypeptide, wherein activity is as defined herein, having at least about 90% amino acid sequence identity with an LP polypeptide having a deduced amino acid sequences as shown above. Such LP polypeptide variants include, for instance, LP polypeptides, wherein one or more amino
20 acid residues are added, substituted or deleted, at the N- or C-terminus or within the sequences shown. Ordinarily, an LP polypeptide variant will have at least about 90% amino acid sequence identity, preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more
25 preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% amino acid sequence identity with the amino acid sequence described, with or without the signal peptide.

30 The term "similar" or "similarity" as used herein describes the relationship between different nucleic acid compounds or amino acid sequences in which said

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sequences or molecules are related by partial sequence identity or sequence similarity at one or more blocks or regions within said molecules or sequences.

In referring to amino acid sequences, the term "similar" or "similarity" describes amino acid residues which are either identical between different amino acid sequences, or
5 represent conservative amino acid substitutions between different sequences.

Conservative amino acid substitutions are listed in Table 1 and discussed infra. The term "identity" describes amino acid residues which are identical between different amino acid sequences. Amino acid sequence similarity or identity with respect to each LP amino acid
10 candidate sequence that are similar or identical with the amino acid residues in an LP polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence similarity or identity.

"Percent (%) amino acid sequence identity" with respect to the LP amino acid sequences identified herein is defined as the percentage of amino acid residues in a
15 candidate sequence that are identical with the amino acid residues in an LP polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art,
20 for instance, using publicly available computer software such as ALIGN, ALIGN-2, Megalign (DNASTAR) or BLAST (e.g., Blast, Blast-2, WU-Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the percent identity values used herein are
25 generated using WU-BLAST-2 [Altschul, *et al.*, *Methods in Enzymology* 266:460-80 (1996)]. Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM 62. For purposes herein, a percent amino acid sequence identity
30 value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the LP polypeptide of interest and the comparison amino acid sequence of interest (i.e., the sequence against which the LP polypeptide of

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interest is being compared) as determined by WU-BLAST-2, by (b) the total number of amino acid residues of the LP polypeptide of interest, respectively.

An "LP variant polynucleotide," "LP polynucleotide variant," or "LP variant nucleic acid sequence" are intended to refer to an nucleic acid molecule as defined below

5 having at least about 75% nucleic acid sequence identity with the polynucleotide sequence as shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23. Ordinarily, an LP polynucleotide variant will have at least about 75% nucleic acid sequence identity, more preferably at least about 80% nucleic acid sequence identity, yet more preferably at least about 81% nucleic acid sequence identity, yet more preferably at least about 82%

10 nucleic acid sequence identity, yet more preferably at least about 83% nucleic acid sequence identity, yet more preferably at least about 84% nucleic acid sequence identity, yet more preferably at least about 85% nucleic acid sequence identity, yet more preferably at least about 86% nucleic acid sequence identity, yet more preferably at least about 87% nucleic acid sequence identity, yet more preferably at least about 88% nucleic

15 acid sequence identity, yet more preferably at least about 89% nucleic acid sequence identity, yet more preferably at least about 90% nucleic acid sequence identity, yet more preferably at least about 91% nucleic acid sequence identity, yet more preferably at least about 92% nucleic acid sequence identity, yet more preferably at least about 93% nucleic acid sequence identity, yet more preferably at least about 94% nucleic acid sequence

20 identity, yet more preferably at least about 95% nucleic acid sequence identity, yet more preferably at least about 96% nucleic acid sequence identity, yet more preferably at least about 97% nucleic acid sequence identity, yet more preferably at least about 98% nucleic acid sequence identity, yet more preferably at least about 99% nucleic acid sequence identity with the nucleic acid sequences shown above. Variants specifically exclude or

25 do not encompass the native nucleotide sequence, as well as those prior art sequences that share 100% identity with the nucleotide sequences of the invention.

"Percent (%) nucleic acid sequence identity" with respect to the LP polynucleotide sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the LP polynucleotide

30 sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in

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the art, for instance, using publicly available computer software such as ALIGN, Align-2, Megalign (DNASTAR), or BLAST (e.g., Blast, Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % nucleic acid identity values are generated using the WU-BLAST-2 (BlastN module) program [Altschul, *et al.*, *Methods in Enzymology* 266:460-80 (1996)]. Most of the WU-BLAST-2 search parameters are set to the default values. Those not set default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM62. For purposes herein, a percent nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the LP polypeptide-encoding nucleic acid molecule of interest and the comparison nucleic acid molecule of interest (i.e., the sequence against which the LP polypeptide-encoding nucleic acid molecule of interest is being compared) as determined by WU-BLAST-2, by (b) the total number of nucleotides of the LP polypeptide-encoding nucleic acid molecule of interest.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul, 1993, Proc. Nat'l Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

In other embodiments, the LP variant polypeptides are encoded by nucleic acid molecules which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding the full-length LP polypeptide as shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24. This scope of variant polynucleotides specifically excludes those sequences that are known as of the filing and/or priority dates of the present application.

The term "homolog" refers to a molecule from a given organism that exhibits sequence similarity and/or identity and has substantially similar biological activity as a molecule from a different organism. For example, a mouse polypeptide that functions in the mouse in a manner equivalent to which human LP391 functions in humans may be referred to as a mouse homolog of LP391. The term "homolog" is also intended to encompass two or more genes or proteins from different organisms or within a single organism that exhibit sequence similarity and/or identity. The term "homolog" as used herein includes allelic variants, as well as splice variants from an LP polynucleotide sequence.

The term "family" as used herein when referring to the polypeptide and nucleic acid molecules of the invention is intended to designate two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence identity and/or similarity as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologs of non-human origin, such as rat or mouse proteins. Members of a family can also have common functional characteristics.

The term "mature protein" or "mature polypeptide" as used herein refers to the form(s) of the protein produced by expression in a mammalian cell. It is generally hypothesized that once export of a growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal peptide (SP) sequence which is cleaved from the complete polypeptide to produce a "mature" form of the protein. Oftentimes, cleavage of a secreted protein is not uniform and may result in more than one species of mature protein. The cleavage site of a secreted protein is determined by the primary amino acid sequence of the complete protein.

Methods for predicting whether a protein has an SP sequence, as well as the cleavage point for that sequence, are known in the art. For example, programs for predicting signal sequences are exemplified by SPScan, SigCleave and SignalP [Menne *et al.*, *Bioinformatics* 16:741-2 (2000)] using information from residues surrounding the cleavage site, typically residues -13 to +2 (where +1 indicates the amino terminus of a secreted protein), or from deduced amino acid sequences of secreted polypeptides. The

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accuracy of predicting cleavage points of known mammalian secretory proteins typically is about 75-80%, however, not all art methods produce the same predicted cleavage point(s) for a given protein. Moreover, cleavage sites can vary from organism to organism and even from molecule to molecule within a cell and cannot be predicted with absolute certainty. Accordingly, the natural positions of the signal cleavage peptide sites and the N-terminal amino acids for the mature LP polypeptides of the present invention may vary from those described herein for LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419, such that the natural amino terminal of the mature polypeptides may contain as many as 5, 4, 3, 2, or 1 additional or fewer amino acids. All such polypeptides and the nucleic acid molecules encoding them are contemplated by the present invention. Optimally, cleavage sites for a secreted protein are determined experimentally by amino-terminal sequencing of the one or more species of mature proteins found within a purified preparation of the protein.

The term "positives," in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g., as a result of conservative substitutions). The percent identity value of positives is determined by the fraction of residues scoring a positive value in the BLOSUM 62 matrix. This value is determined by dividing (a) the number of amino acid residues scoring a positive value in the BLOSUM62 matrix of WU-BLAST-2 between the LP polypeptide amino acid sequence of interest and the comparison amino acid sequence (i.e., the amino acid sequence against which the LP polypeptide sequence is being compared) as determined by WU-BLAST-2, by (b) the total number of amino acid residues of the LP polypeptide of interest.

"Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to

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homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the LP polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated LP polypeptide-encoding nucleic acid" or "isolated LP nucleic acid" is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid. Such an isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated LP polypeptide-encoding nucleic acid molecule includes LP polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express LP polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adapters or linkers are used in accordance with conventional practice.

The term "amino acid" is used herein in its broadest sense, and includes naturally occurring amino acids as well as non-naturally occurring amino acids, including amino acid analogs and derivatives. The latter includes molecules containing an amino acid moiety. One skilled in the art will recognize, in view of this broad definition, that reference herein to an amino acid includes, for example, naturally occurring proteogenic L-amino acids; D-amino acids; chemically modified amino acids such as amino acid

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analogs and derivatives; naturally-occurring non-proteogenic amino acids such as norleucine, beta-alanine, ornithine, etc.; and chemically synthesized compounds having properties known in the art to be characteristic of amino acids. As used herein, the term “proteogenic” indicates that the amino acid can be incorporated into a peptide, polypeptide, or protein in a cell through a metabolic pathway.

The incorporation of non-natural amino acids, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the LP peptides, polypeptides, or proteins of the present invention (“D-LP polypeptides”) is advantageous in a number of different ways. D-amino acid-containing peptides, polypeptides, or proteins exhibit increased stability *in vitro* or *in vivo* compared to L-amino acid-containing counterparts. Thus, the construction of peptides, polypeptides, or proteins incorporating D-amino acids can be particularly useful when greater intracellular stability is desired or required. More specifically, D-peptides, polypeptides, or proteins are resistant to endogenous peptidases and proteases, thereby providing improved bioavailability of the molecule and prolonged lifetimes *in vivo* when such properties are desirable. When it is desirable to allow the peptide, polypeptide, or protein to remain active for only a short period of time, the use of L-amino acids therein will permit endogenous peptidases, proteases, etc., in a cell to digest the molecule *in vivo*, thereby limiting the cell’s exposure to the molecule. Additionally, D-peptides, polypeptides, or proteins cannot be processed efficiently for major histocompatibility complex class II-restricted presentation to T helper cells, and are therefore less likely to induce humoral immune responses in the whole organism.

In addition to using D-amino acids, those of ordinary skill in the art are aware that modifications in the amino acid sequence of a peptide, polypeptide, or protein can result in equivalent, or possibly improved, second generation peptides, polypeptides, or proteins, that display equivalent or superior functional characteristics when compared to the original amino acid sequences. Alterations in the LP peptides, polypeptides, or proteins of the present invention can include one or more amino acid insertions, deletions, substitutions, truncations, fusions, shuffling of subunit sequences, and the like, either from natural mutations or human manipulation, provided that the sequences produced by such modifications have substantially the same (or improved or reduced, as

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may be desirable) activity(ies) as the naturally-occurring counterpart sequences disclosed herein.

One factor that can be considered in making such changes is the hydropathic index of amino acids. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein has been discussed by Kyte and Doolittle [*J. Mol. Biol.* 157:105-32 (1982)]. It is accepted that the relative hydropathic character of amino acids contributes to the secondary structure of the resultant protein. This, in turn, affects the interaction of the protein with molecules such as enzymes, substrates, receptors, ligands, DNA, antibodies, antigens, etc. Based on its hydrophobicity and charge characteristics, each amino acid has been assigned a hydropathic index as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate/glutamine/aspartate/asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

As is known in the art, certain amino acids in a peptide, polypeptide, or protein can be substituted for other amino acids having a similar hydropathic index or score and produce a resultant peptide, polypeptide, or protein having similar biological activity, i.e., which still retains biological functionality. In making such changes, it is preferable that amino acids having hydropathic indices within ± 2 are substituted for one another. More preferred substitutions are those wherein the amino acids have hydropathic indices within ± 1 . Most preferred substitutions are those wherein the amino acids have hydropathic indices within ± 0.5 .

Like amino acids can also be substituted on the basis of hydrophilicity. U.S. Patent 4,554,101 discloses that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. The following hydrophilicity values have been assigned to amino acids: arginine/lysine (+3.0); aspartate/glutamate (+3.0 \pm 1); serine (+0.3); asparagine/glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). Thus, one amino acid in a peptide, polypeptide, or protein can be substituted by another

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amino acid having a similar hydrophilicity score and still produce a resultant peptide, polypeptide, or protein having similar biological activity, i.e., still retaining correct biological function. In making such changes, amino acids having hydrophobic indices within ± 2 are preferably substituted for one another, those within ± 1 are more preferred, and those within ± 0.5 are most preferred.

As outlined above, amino acid substitutions in the LP polypeptides of the present invention can be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, etc. Exemplary substitutions that take various of the foregoing characteristics into consideration in order to produce conservative amino acid changes resulting in silent changes within the present peptides, polypeptides, or proteins can be selected from other members of the class to which the naturally occurring amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids; (2) basic amino acids; (3) neutral polar amino acids; and (4) neutral non-polar amino acids. Representative amino acids within these various groups include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral non-polar amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

It should be noted that changes which are not expected to be advantageous can also be useful if these result in the production of functional sequences. Since small peptides, polypeptides, and some proteins can be easily produced by conventional solid phase synthetic techniques, the present invention includes peptides, polypeptides, or proteins such as those discussed herein, containing the amino acid modifications discussed above, alone or in various combinations. To the extent that such modifications can be made while substantially retaining the activity of the peptide, polypeptide, or protein, they are included within the scope of the present invention. The utility of such modified peptides, polypeptides, or proteins can be determined without undue experimentation by, for example, the methods described herein.

While biologically functional equivalents of the present LP polypeptides can have any number of conservative or non-conservative amino acid changes that do not

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significantly affect their activity(ies), or that increase or decrease activity as desired, the number of changes preferred may be 40, 30, 20, 10, 5, 3 or fewer changes, or any range or value therein, such as 1 to 30 changes. In particular, ten or fewer amino acid changes may be preferred. More preferably, seven or fewer amino acid changes may be preferred; most preferably, five or fewer amino acid changes may be preferred. The encoding nucleotide sequences (gene, plasmid DNA, cDNA, synthetic DNA, or mRNA, for example) will thus have corresponding base substitutions, permitting them to code expression for the biologically functional equivalent forms of the LP polypeptides. In any case, the LP peptides, polypeptides, or proteins exhibit the same or similar biological or immunological activity(ies) as that(those) of the LP polypeptides specifically disclosed herein, or increased or reduced activity, if desired. The activity(ies) of the variant LP polypeptides can be determined by the methods described herein. Variant LP polypeptides biologically functionally equivalent to those specifically disclosed herein have activity(ies) differing from those of the presently disclosed molecules by about $\pm 50\%$ or less, preferably by about $\pm 40\%$ or less, more preferably by about $\pm 30\%$ or less, more preferably by about $\pm 20\%$ or less, and even more preferably by about $\pm 10\%$ or less, when assayed by the methods disclosed herein.

Amino acids in an LP polypeptide of the present invention that are essential for activity can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis [Cunningham and Wells, *Science* 244:1081-5 (1989)]. The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity. Sites that are critical for ligand-protein binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance, or photoaffinity labeling [Smith, *et al.*, *J. Mol. Biol.* 224:899-904 (1992); de Vos, *et al.*, *Science* 255:306-12 (1992)].

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer nucleic acid probes required higher temperatures for proper annealing, while shorter nucleic acid probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired complementarity between the probe and

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hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reactions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel, *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers (1995).

“Stringent conditions” or “high stringency conditions,” as defined herein, may be identified by those that (1) employ low ionic strength and high temperature for washing, for example, 15 mM sodium chloride/1.5 mM sodium citrate/0.1% sodium dodecyl sulfate at 50 degrees C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride/75 mM sodium citrate at 42 degrees C; or (3) employ 50% formamide, 5X SSC (750 mM sodium chloride, 75 mM sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt’s solution, sonicated salmon sperm DNA (50 µg/mL), 0.1% SDS, and 10% dextran sulfate at 42 degrees C with washes at 42 degrees C in 0.2X SSC (30 mM sodium chloride/3 mM sodium citrate) and 50% formamide at 55 degrees C, followed by a high-stringency wash consisting of 0.1X SSC containing EDTA at 55 degrees C.

“Moderately stringent conditions” may be identified as described by Sambrook, *et al.* [*Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, (1989)], and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37 degrees C in a solution comprising: 20% formamide, 5X SSC (750 mM sodium chloride, 75 mM sodium citrate), 50 mM sodium phosphate at pH 7.6, 5X Denhardt’s solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1X SSC at about 37 to 50 degrees C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc., as necessary to accommodate factors such as probe length and the like.

The term “epitope tagged” where used herein refers to a chimeric polypeptide comprising an LP polypeptide, or domain sequence thereof, fused to a “tag polypeptide.” The tag polypeptide has enough residues to provide an epitope against which an antibody

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may be made, or which can be identified by some other agent, yet is short enough such that it does not interfere with the activity of the LP polypeptide. The tag polypeptide preferably is also fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about eight to about fifty amino acid residues (preferably, between about ten to about twenty residues).

As used herein, the term "immunoadhesin," sometimes referred to as an Fc fusion, designates antibody-like molecules that combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

"Active" or "activity" for the purposes herein refers to form(s) of LP polypeptide which retain all or a portion of the biologic and/or immunologic activities of native or naturally-occurring LP polypeptide. Elaborating further, "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring LP polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring LP polypeptide. An "immunological" activity refers only to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring LP polypeptide.

The term "antagonist" is used in the broadest sense and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native LP polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native LP polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid

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sequence variants of native LP polypeptides, peptides, ribozymes, anti-sense nucleic acids, small organic molecules, etc. Methods for identifying agonists or antagonists of an LP polypeptide may comprise contacting an LP polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the LP polypeptide.

“Antibodies” (Abs) and “immunoglobulins” (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term “antibody” is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

The terms “treating,” “treatment,” and “therapy” as used herein refer to curative therapy, prophylactic therapy, and preventive therapy. An example of “preventive therapy” is the prevention or lessened targeted pathological condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

“Chronic” administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. “Intermittent” administration is treatment that is not consecutively done without interruption but, rather, is cyclic in nature.

Administration “in combination with” one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

A “therapeutically-effective amount” is the minimal amount of active agent (e.g., an LP polypeptide, antagonist or agonist thereof) which is necessary to impart therapeutic benefit to a mammal. For example, a “therapeutically-effective amount” to a mammal suffering or prone to suffering or to prevent it from suffering is such an amount which induces, ameliorates, or otherwise causes an improvement in the pathological symptoms, disease progression, physiological conditions associated with or resistance to succumbing to the aforescribed disorder.

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“Carriers” as used herein include pharmaceutically-acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically-acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecule weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONIC®.

“Antibody fragments” comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies [Zapata, *et al.*, *Protein Engin.* 8 (10):1057-62 (1995)]; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

“Fv” is the minimum antibody fragment which contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_HV_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

“Single-chain Fv” or “sFv” antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domain, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun, *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore, eds., Springer-Verlag, New York, pp. 269-315 (1994).

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The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404 097; WO 93/11161; and Hollinger, *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-8 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue, or preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An "LP polypeptide antibody" or "LP antibody" refers to an antibody as defined herein that recognizes and binds at least one epitope of an LP polypeptide of the present invention. The term "LP polypeptide antibody" or "LP antibody" wherein the term "LP" is followed by a numerical designation refers to an antibody that recognizes and binds to at least one epitope of that particular LP polypeptide as disclosed herein.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an LP polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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A "small molecule" is defined herein to have a molecular weight below about 500 daltons.

The term "modulate" means to affect (e.g., either upregulate, downregulate or otherwise control) the level of a signaling pathway. Cellular processes under the control of signal transduction include, but are not limited to, transcription of specific genes, normal cellular functions, such as metabolism, proliferation, differentiation, adhesion, apoptosis and survival, as well as abnormal processes, such as transformation, blocking of differentiation and metastasis.

An LP polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the LP polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, LP polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. LP polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

LP polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the gene-encoded amino acids. The LP polypeptides may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the LP polypeptides, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given LP polypeptide. Also, a given LP polypeptide may contain many types of modifications. LP polypeptides may be branched, for

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example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic LP polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, Creighton, *Proteins – Structure and Molecular Properties*, 2nd Ed.; W. H. Freeman and Company, New York (1993); Johnson, *Post-translational Covalent Modification of Proteins*, Academic Press, New York, pp. 1-12 (1983); Seifter, *et al.*, *Meth. Enzymol.* 182:626-46 (1990); Rattan, *et al.*, *Ann. NY Acad. Sci.* 663:48-62 (1992).

Variations in the full-length sequence LP polypeptide or in various domains of the LP polypeptide described herein can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding LP polypeptide that results in a change in the amino acid sequence of the LP polypeptide as compared with the native sequence LP polypeptide or an LP polypeptide as disclosed herein. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the LP polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the LP polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high identity and/or similarity. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of one to five

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amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity (such as in any of the *in vitro* assays described herein) for activity exhibited by the full-length or mature polypeptide sequence.

5 LP polypeptide fragments are also provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length or native protein. Certain fragments contemplated by the present invention may lack amino acid residues that are not essential for a desired biological activity of the LP polypeptide.

10 LP polypeptide fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating LP fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the
15 desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, LP polypeptide fragments share at least one biological and/or immunological activity with at least one of the LP
20 polypeptides as shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

Covalent modifications of LP polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of an LP polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of an LP polypeptide.

25 Derivatization with bifunctional agents is useful, for instance, for crosslinking LP polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-LP polypeptide antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid,
30 homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis-(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithiolpropionimide].

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Other modifications include deamidation of glutamyl and asparagyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains
5 [Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the LP polypeptides included within the scope of this invention comprises altering the native glycosylation pattern of the
10 polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence LP polypeptide and/or adding one or more glycosylation sites that are not present in the native sequences of LP polypeptides. Additionally, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and
15 proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to LP polypeptides may be accomplished by altering the amino acid sequence thereof. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequences of LP polypeptides (for O-linked glycosylation sites). The LP amino acid
20 sequences may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the LP polypeptides at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the LP polypeptides is by chemical or enzymatic coupling of glycosides to the polypeptide.
25 Such methods are described in the art, e.g., in WO 87/05330, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the LP polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical
30 deglycosylation techniques are known in the art and described, for instance, by Sojar, *et al.*, *Arch. Biochem. Biophys.* 259:52-7 (1987), and by Edge, *et al.*, *Anal. Biochem.* 118:131-7 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be

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achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura, *et al.*, *Meth. Enzymol.* 138:350-9 (1987).

Another type of covalent modification of LP comprises linking any one of the LP polypeptides to one of a variety of non-proteinaceous polymers (e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes) in the manner set forth in U.S. Patent
5 Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192, or 4,179,337.

LP polypeptides of the present invention may also be modified in a way to form chimeric molecules comprising an LP polypeptide fused to another heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule
10 comprises a fusion of an LP polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of LP359, LP364, LP381, LP400, or LP401 polypeptide. The presence of such epitome-tagged forms of an LP polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitome
15 tag enables an LP polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitome tag.

In an alternative embodiment, the chimeric molecule may comprise a fusion of an LP polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of
20 an IgG molecule. The Ig fusions preferably include the substitution of a soluble transmembrane domain deleted or inactivated form of an LP polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3 or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions,
25 see also U.S. Patent 5,428,130.

In yet a further embodiment, the LP polypeptides of the present invention may also be modified in a way to form a chimeric molecule comprising an LP polypeptide fused to a leucine zipper. Various leucine zipper polypeptides have been described in the art. See, e.g., Landschulz, *et al.*, *Science* 240(4860):1759-64 (1988); WO 94/10308;
30 Hoppe, *et al.*, *FEBS Letters* 344(2-3):191-5 (1994); Abel, *et al.*, *Nature* 341(6237):24-5 (1989). It is believed that use of a leucine zipper fused to an LP polypeptide may be desirable to assist in dimerizing or trimerizing soluble LP polypeptide in solution. Those

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skilled in the art will appreciate that the zipper may be fused at either the N- or C-terminal end of an LP polypeptide.

The description below relates primarily to production of LP polypeptides by culturing cells transformed or transfected with a vector containing an LP polypeptide-
5 encoding nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare LP polypeptides. For instance, the LP polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart, *et al.*, *Solid-Phase Peptide Synthesis*, W.H. Freeman & Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-
10 2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of an LP polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce a full-length
15 LP polypeptide.

DNA encoding an LP polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the LP polypeptide-encoding mRNA and to express it at a detectable level. Libraries can be screened with probes (such as antibodies to an LP polypeptide or oligonucleotides of at least about 20 to 80 bases) designed to identify the
20 gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY (1989). An alternative means to isolate the gene encoding an LP polypeptide is to use PCR methodology [Sambrook, *et al.*, *supra*; Dieffenbach, *et al.*,
25 *PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY (1995)].

Nucleic acids having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time and, if necessary, using conventional primer extension procedures as described in Sambrook, *et al.*, *supra*, to detect precursors and processing intermediates
30 of mRNA that may not have been reverse-transcribed into cDNA.

Host cells are transfected or transformed with expression or cloning vectors described herein for LP polypeptide production and cultured in conventional nutrient

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media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell*
5 *Biotechnology: A Practical Approach*, Butler, ed. (IRL Press, 1991) and Sambrook, *et al.*, *supra*. Methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate and electroporation. General aspects of mammalian cell host system transformations have been described in U.S. Patent 4,399,216.

10 Transformations into yeast are typically carried out according to the method of van Solingen, *et al.*, *J. Bact.* 130(2):946-7 (1977) and Hsiao, *et al.*, *Proc. Natl. Acad. Sci. USA* 76(8):3829-33 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene or polyornithine, may also be used. For various
15 techniques for transforming mammalian cells, see Keown, *et al.*, *Meths. in Enzymology* 185:527-37 (1990) and Mansour, *et al.*, *Nature* 336(6197):348-52 (1988).

Suitable host cells for cloning or expressing the nucleic acid (e.g., DNA) in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive
20 organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* strain X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g.,
25 *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescens*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710, published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for
30 recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3 110 may be modified to effect a genetic mutation in a gene encoding proteins endogenous to the host, with examples of

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such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype tonAD; *E. coli* W3110 strain 9E4, which has the complete genotype tonAD ptr3; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype tonAD ptr3 phoADE15 D(argF-lac)169 ompTD degP41kan^{R'}; *E. coli* W3110 strain 37D6, which has the complete genotype tonAD ptr3 phoADE15 D(argF-lac)169 ompTD degP41kan^R rbs7D ilvG; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant degP deletion mutation; and an *E. coli* strain having mutant periplasmic protease as disclosed in U.S. Patent 4,946,783 issued 7 August 1990. Alternatively, *in vivo* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for LP vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* [Beach and Nurse, *Nature* 290:140-3 (1981); EP 139,383 published 2 May 1995]; *Muyveromyces* hosts [U.S. Patent 4,943,529 Fleer, *et al.*, *Bio/Technology* 9(10):968-75 (1991)] such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574) [de Louvencourt, *et al.*, *J. Bacteriol.* 154(2):737-42 (1983)]; *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36.906) [Van den Berg, *et al.*, *Bio/Technology* 8(2):135-9 (1990)]; *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070) [Sreekrishna, *et al.*, *J. Basic Microbiol.* 28(4):265-78 (1988)]; *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa* [Case, *et al.*, *Proc. Natl. Acad. Sci. USA* 76(10):5259-63 (1979)]; *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* [Ballance, *et al.*, *Biochem. Biophys. Res. Comm.* 112(1):284-9 (1983); Tilburn, *et al.*, *Gene* 26(2-3):205-21 (1983); Yelton, *et al.*, *Proc. Natl. Acad. Sci. USA* 81(5):1470-4 (1984)] and *A. niger* [Kelly and Hynes, *EMBO J.* 4(2):475-9 (1985)]. Methylotropic yeasts are selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and

Rhodotoruia. A list of specific species that are exemplary of this class of yeast may be found in Antony, *The Biochemistry of Methylophilos* 269 (1982).

Suitable host cells for the expression of glycosylated LP polypeptides are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sp, *Spodoptera* high5 as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line [293 or 293 cells subcloned for growth in suspension culture, Graham, *et al.*, *J. Gen Virol.*, 36(1):59-74 (1977)]; Chinese hamster ovary cells/-DHFR [CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77(7):4216-20 (1980)]; mouse sertoli cells [TM4, Mather, *Biol. Reprod.* 23(1):243-52 (1980)]; human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

LP polypeptides may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the LP polypeptide-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* cc-factor leaders, the latter described in U.S. Patent 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179), or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for

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yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the LP polypeptide-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77(7):4216-20 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb, *et al.*, *Nature* 282(5734):39-43 (1979); Kingsman, *et al.*, *Gene* 7(2):141-52 (1979); Tschumper, *et al.*, *Gene* 10(2):157-66 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEPC1 [Jones, *Genetics* 85:23-33 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the LP polypeptide-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the P-lactamase and lactose promoter systems [Chang, *et al.*, *Nature* 275(5681):617-24 (1978); Goeddel, *et al.*, *Nature* 281(5732):544-8 (1979)], alkaline phosphatase, a tryptophan (up) promoter system [Goeddel, *Nucleic Acids Res.* 8(18):4057-74 (1980); EP 36,776 published 30 September 1981], and hybrid promoters such as the *tat* promoter [deBoer, *et al.*, *Proc. Natl. Acad. Sci. USA* 80(1):21-5 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding LP polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman, *et al.*, *J. Biol. Chem.* 255(24):12073-80 (1980)] or other glycolytic enzymes [Hess, *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); Holland, *Biochemistry* 17(23):4900-7 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase,

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phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for
5 alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. LP transcription from vectors in mammalian host cells is controlled, for example, by
10 promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with
15 the host cell systems.

Transcription of a polynucleotide encoding an LP polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from
20 mammalian genes (globin, elastase, albumin, alpha-ketoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the
25 vector at a position 5' or 3' to the LP polypeptide coding sequence but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA.
30 Such sequences are commonly available from the 5' and occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide

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segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding LP.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA* 77(9):5201-5 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence provided herein or against exogenous sequence fused to an LP polypeptide-encoding DNA and encoding a specific antibody epitope.

Various forms of an LP polypeptide may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g., Triton X-100TM) or by enzymatic cleavage. Cells employed in expression of an LP polypeptide can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desirable to purify LP polypeptides from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reversed-phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex[®] G-75; protein A Sepharose[®] columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of an LP polypeptide.

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Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, *Methods in Enzymology* 182:83-9 (1990) and Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, NY (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular LP polypeptide produced.

Nucleotide sequences (or their complement) encoding LP polypeptides have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. LP polypeptide-encoding nucleic acids will also be useful for the preparation of LP polypeptides by the recombinant techniques described herein.

The full-length LP polypeptide-encoding nucleotide sequence (e.g., SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23), or portions thereof, may be useful as hybridization probes for probing a cDNA or genomic library to isolate the full-length LP polypeptide-encoding cDNA or genomic sequences including promoters, enhancer elements and introns of native sequence LP polypeptide-encoding DNA or to isolate still other genes (for instance, those encoding naturally-occurring variants of LP polypeptides or the same from other species) which have a desired sequence identity to the LP polypeptide-encoding nucleotide sequence disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23. Hybridization techniques are well known in the art and some of which are described in further detail in the Examples below.

Other useful fragments of the LP polypeptide-encoding nucleic acids include anti-sense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target LP polypeptide-encoding mRNA (sense) or LP polypeptide-encoding DNA (anti-sense) sequences. Anti-sense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of LP polypeptide-encoding DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an anti-sense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48(10):2659-68 (1988) and van der Krol, *et al.*, *Bio/Techniques* 6(10):958-76 (1988).

Binding of anti-sense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target

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sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The anti-sense oligonucleotides thus may be used to block expression of LP mRNA and therefore any LP polypeptide encoded thereby. Anti-sense or sense oligonucleotides further comprise
5 oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

10 Other examples of sense or anti-sense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such poly-L-lysine. Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or anti-
15 sense oligonucleotides to modify binding specificities of the anti-sense or sense oligonucleotide for the target nucleotide sequence.

Anti-sense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, calcium phosphate-mediated DNA transfection, electroporation, or by using gene transfer
20 vectors such as Epstein-Barr virus. In a preferred procedure, an anti-sense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MSV, N2 (a retrovirus derived from M-MuLV), or the double copy
25 vectors designated CDTSA, CTSB and DCTSC (see WO 90/13641).

Alternatively, a sense or an anti-sense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or anti-sense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

30 When the amino acid sequence for an LP polypeptide encodes a protein which binds to another protein (for example, where the LP polypeptide functions as a receptor), the LP polypeptide can be used in assays to identify the other proteins or molecules

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involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor LP polypeptide can be used to isolate correlative ligand(s).

5 Screening assays can be designed to find lead compounds that mimic the biological activity of the LP polypeptides disclosed herein or a receptor for such LP polypeptides. Typical screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic
10 compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode an LP polypeptide of the present invention or any of its modified forms can also be used to generate either transgenic animals or "knockout"
15 animals which, in turn, are useful in the development and screening of therapeutically useful reagents. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patents 4,736,866 and 4,870,009. Typically, particular cells would be targeted for an LP transgene incorporation with tissue-specific enhancers. Transgenic animals that include a
20 copy of a transgene introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding an LP polypeptide. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a
25 reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologs of LP polynucleotides can be used to construct a "knockout" animal which has a defective or altered gene encoding a particular
30 LP polypeptide as a result of homologous recombination between the endogenous gene encoding the LP polypeptide and the altered genomic DNA introduced into an embryonic cell of the animal. For example, cDNA encoding an LP polypeptide can be used to clone

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genomic DNA encoding that LP polypeptide in accordance with established techniques. A portion of the genomic DNA encoding an LP polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see, e.g., Thomas and Capecchi, *Cell* 51(3):503-12 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation), and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see, e.g., Li, *et al.*, *Cell* 69(6):915-26 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see, e.g., Bradley, *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed., pp. 113-152 (IRL, Oxford, 1987)]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knockout" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized, for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the native LP polypeptide.

Transgenic non-human mammals are useful as animal models in both basic research and drug development endeavors. Transgenic animals expressing at least one LP polypeptide or nucleic acid can be used to test compounds or other treatment modalities which may prevent, suppress, or cure a pathology or disease associated with at least one of the above mentioned activities. Such transgenic animals can also serve as a model for the testing of diagnostic methods for those same diseases. Furthermore, tissues derived from such transgenic non-human mammals are useful as a source of cells for cell culture in efforts to develop *in vitro* bioassays to identify compounds that modulate LP polypeptide activity or LP polypeptide dependent signaling. Accordingly, another aspect of the present invention contemplates a method of identifying compounds efficacious in the treatment of at least one previously described disease or pathology associated with an LP polypeptide associated activity. A non-limiting example of such a method comprises:

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a) generating a transgenic non-human animal which expresses an LP polypeptide of the present invention and which is, as compared to a wild-type animal, pathologically distinct in some detectable or measurable manner from wild-type version of said non-human mammal;

5 b) exposing said transgenic animal to a compound, and;

c) determining the progression of the pathology in the treated transgenic animal, wherein an arrest, delay, or reversal in disease progression in transgenic animal treated with said compound as compared to the progression of the pathology in an untreated control animals is indicative that the compound is useful for the treatment of said
10 pathology.

Another embodiment of the present invention provides a method of identifying compounds capable of inhibiting LP polypeptide activity *in vivo* and/or *in vitro* wherein said method comprises:

a) administering an experimental compound to an LP polypeptide expressing
15 transgenic non-human animal, or tissues derived therefrom, exhibiting one or more physiological or pathological conditions attributable to the expression of an LP transgene; and

b) observing or assaying said animal and/or animal tissues to detect changes in said physiological or pathological condition or conditions.

20 Another embodiment of the invention provides a method for identifying compounds capable of overcoming deficiencies in LP polypeptide activity *in vivo* or *in vitro* wherein said method comprises:

a) administering an experimental compound to an LP polypeptide expressing
25 transgenic non-human animal, or tissues derived therefrom, exhibiting one or more physiological or pathological conditions attributable to the disruption of the endogenous LP polypeptide-encoding gene; and

b) observing or assaying said animal and/or animal tissues to detect changes in said physiological or pathological condition or conditions.

30 Various means for determining a compound's ability to modulate the activity of an LP polypeptide in the body of the transgenic animal are consistent with the invention. Observing the reversal of a pathological condition in the LP polypeptide expressing transgenic animal after administering a compound is one such means. Another more

preferred means is to assay for markers of LP activity in the blood of a transgenic animal before and after administering an experimental compound to the animal. The level of skill of an artisan in the relevant arts readily provides the practitioner with numerous methods for assaying physiological changes related to therapeutic modulation of LP activity.

“Gene therapy” includes both conventional gene therapy, where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Anti-sense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short anti-sense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane [Zamecnik, *et al.*, *Proc. Natl. Acad. Sci. USA* 83(12):4143-6 (1986)]. The oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups with uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cell *in vitro* or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically, retroviral) vectors and viral coat protein-liposome mediated transfection [Dzau, *et al.*, *Trends in Biotechnology* 11(5):205-10 (1993)]. In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cells, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof trophic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu, *et al.*, *J. Biol. Chem.* 262(10):4429-32 (1987); and Wagner, *et al.*, *Proc.*

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Natl. Acad. Sci. USA 87(9):3410-4 (1990). For a review of gene marking and gene therapy protocols, see Anderson, *Science* 256(5058):808-13 (1992).

The nucleic acid molecules encoding LP polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data, are presently available. Each LP polypeptide-encoding nucleic acid molecule of the present invention can be used as a chromosome marker. An LP polypeptide-encoding nucleic acid or fragments thereof can also be used for chromosomal localization of the gene encoding that LP polypeptide.

The present invention further provides anti-LP polypeptide antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

The anti-LP polypeptide antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the LP polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized.

Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The immunization protocol may be selected by one skilled in the art without undue experimentation.

The anti-LP polypeptide antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature* 256(5517):495-7 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing

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antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include an LP polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used, if cells of human origin are desired, or spleen cells or lymph node cells are used, if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, CA, and the American Type Culture Collection (ATCC), Rockville, MD. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.* 133(6):3001-5 (1984); Brodeur, *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., NY, pp. 51-63 (1987)].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against an LP polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal

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antibody can, for example, be determined by the Scatchard analysis of Munson and Rodbard, *Anal. Biochem.* 107(1):220-39 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent 4,816,567; Morrison, *et al.*, *Proc. Natl. Acad. Sci. USA* 81(21):6851-5 (1984)] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

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In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

The anti-LP polypeptide antibodies of the invention may further comprise
5 humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which
10 residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found
15 neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized
20 antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones, *et al.*, *Nature* 321(6069):522-5 (1986); Riechmann, *et al.*, *Nature* 332(6162):323-7 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-6 (1992)].

Methods for humanizing non-human antibodies are well known in the art.
25 Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones, *et al.*, *Nature* 321(6069):522-5 (1986); Riechmann, *et al.*, *Nature*
30 332(6162):323-7 (1988); Verhoeven, *et al.*, *Science* 239(4847):1534-6 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S.

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Patent 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.* 227(2):381-8 (1992); Marks, *et al.*, *J. Mol. Biol.* 222(3):581-97 (1991)]. The techniques of Cole, *et al.*, and Boerner, *et al.*, are also available for the preparation of human monoclonal antibodies (Cole, *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985), and Boerner, *et al.*, *J. Immunol.* 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patents 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks, *et al.*, *Biotechnology* 10(7):779-83 (1992); Lonberg, *et al.*, *Nature* 368(6474):856-9 (1994); Morrison, *Nature* 368(6474):812-3 (1994); Fishwild, *et al.*, *Nature Biotechnology* 14(7):845-51 (1996); Neuberger, *Nature Biotechnology* 14(7):826 (1996); Lonberg and Huszar, *Int. Rev. Immunol.* 13(1):65-93 (1995).

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an LP polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit. Methods for making bispecific antibodies are known in the art. Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared [Tutt, *et al.*, *J Immunol.* 147(1):60-9 (1991)].

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted

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cells [U.S. Patent 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/20373]. It is contemplated that the antibodies may be prepared *in-vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange
5 reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent 4,676,980.

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an
10 enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof, or a small molecule toxin), or a radioactive isotope (i.e., a radioconjugate).

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as
15 dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds, bis-diazonium derivatives (such as bis-2-diazoniumbenzoyl)-ethylenediamine) diisocyanates (such as tolylene-2,6-diisocyanate), and bioactive fluorine compounds. For example, a ricin immunotoxin can be prepared as described in Vitetta, *et al.*, *Science* 238(4830):1098-104 (1987). Carbon-14-labeled 1-
20 isothiocyantobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the
25 circulation using a clearing agent, and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Eppstein, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-92 (1985); Hwang, *et al.*, *Proc. Natl. Acad. Sci. USA* 77(7):4030-4 (1980); and U.S. Patents 4,485,045 and
30 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent 5,013,556.

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Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin, *et al.*, *J. Biol. Chem.* 257(1):286-8 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon, *et al.*, *J. National Cancer Inst.* 81(19):484-8 (1989).

Antibodies specifically binding an LP polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If an LP polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody or an antibody fragment into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco, *et al.*, *Proc. Natl. Acad. Sci. USA* 90(16):7889-93 (1993).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokines, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitable present in combination in amounts that are effective for the purpose intended. The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and

nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

5 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol), polylactides (U.S. Patent 3,773,919), copolymers of L-glutamic acid gamma-ethyl-L-glutamate, non-degradable ethylene-vinylacetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 degrees C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanisms involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thiosulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

25 The anti-LP polypeptide antibodies of the present invention have various utilities. For example, such antibodies may be used in diagnostic assays for LP polypeptide expression, e.g., detecting expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc., pp. 147-158 (1987)]. The antibodies used in the assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable

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moiety may be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter, *et al.*, *Nature* 144:945 (1962); David, *et al.*, *Biochemistry* 13(5):1014-21 (1974); Pain, *et al.*, *J Immunol. Meth.*, 40(2):219-30 (1981); and Nygren, *J. Histochem. Cytochem.* 30(5):407-12 (1982).

Anti-LP polypeptide antibodies also are useful for affinity purification from recombinant cell culture or natural sources. In this process, the antibodies are immobilized on a suitable support, such a Sephadex[®] resin or filter paper, using methods well known in the art. The immobilized antibody is then contacted with a sample containing the LP polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the LP polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the desired polypeptide from the antibody.

This invention encompasses methods of screening compounds to identify those that mimic the activity of the LP polypeptide (agonists) disclosed herein or prevent the effects of the LP polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with an LP polypeptide encoded by the genes identified herein or otherwise interfere with the interaction of LP polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats. In binding assays, the interaction is binding, and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, an LP polypeptide encoded by a gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution comprising LP polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a

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detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection
5 of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to an LP polypeptide,
10 its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored through gradients or chromatographic columns. In addition, protein-protein interactions
15 can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature* 340(6230):245-6 (1989); Chien, *et al.*, *Proc. Natl. Acad. Sci. USA* 88(21):9578-82 (1991); Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89(13):5789-93 (1992)]. Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding
20 domain, while the other functions as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another in which candidate activating proteins are fused to the activation domain. The
25 expression of GAL1-lacZ reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with chromogenic substrate for beta-galactosidase. A complete kit (MATCHMAKERTM) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is
30 commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

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Compounds that interfere with the interaction of an LP polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture to serve as a positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove.

10 The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

Antagonists may be detected by combining at least one LP polypeptide and a potential antagonist with a membrane-bound or recombinant receptor for that LP polypeptide under appropriate conditions for a competitive inhibition assay. The LP polypeptide can be labeled, such as by radioactivity, such that the number of LP polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor for an LP polypeptide can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. See Coligan, *et al.*, *Current Protocols in Immunology* 1(2):Ch. 5 (1991). Preferably, expression cloning is employed such that polyadenylated mRNA is prepared from a cell responsive to the secreted form of a particular LP polypeptide, and a cDNA library created from this mRNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the secreted LP polypeptide. Transfected cells that are grown on glass slides are exposed to the labeled LP polypeptide. The LP polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

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As an alternative approach for receptor identification, a labeled LP polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide
5 fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation
10 expressing the receptor would be incubated with a labeled LP polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be removed.

Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the LP polypeptide that recognizes the receptor but imparts no effect,
15 thereby competitively inhibiting the action of the polypeptide.

Another potential LP antagonist is an anti-sense RNA or DNA construct prepared using anti-sense technology, where, e.g., an anti-sense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and prevent its translation into protein. Anti-sense technology can be used to control gene expression
20 through triple-helix formation or anti-sense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature form of an LP polypeptide can be used to design an anti-sense RNA oligonucleotide sequence of about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary
25 to a region of the gene involved in transcription [triple helix; see Lee, *et al.*, *Nucl. Acids Res* 6(9):3073-91 (1979); Cooney, *et al.*, *Science* 241(4864):456-9 (1988); Beal and Dervan, *Science* 251(4999):1360-3 (1991)], thereby preventing transcription and production of the LP polypeptide. The anti-sense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecules [anti-sense; see Okano, *J. Neurochem.* 56(2):560-7 (1991); *Oligodeoxynucleotides as Anti-sense Inhibitors of Gene Expression* (CRC Press: Boca Raton, FL 1988)]. The oligonucleotides described above
30 can also be delivered to cells such that the anti-sense RNA or DNA may be expressed *in*

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vivo to inhibit production of the LP polypeptide. When anti-sense DNA is used, oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the LP polypeptide, thereby blocking the normal biological activity of the LP polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details, see, e.g., Rossi, *Current Biology* 4(5):469-71 (1994) and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

Another use of the compounds of the invention (e.g., LP polypeptides, fragments and variants thereof and LP antibodies directed thereto) described herein is to help diagnose whether a disorder is driven to some extent by the modulation of signaling by an LP polypeptide

A diagnostic assay to determine whether a particular disorder is driven by LP polypeptide dependent signaling can be carried out using the following steps:

- a) culturing test cells or tissues expressing an LP polypeptide;
- b) administering a compound which can inhibit LP polypeptide dependent signaling;
- and
- c) measuring LP polypeptide mediated phenotypic effects in the test cells.

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The steps can be carried out using standard techniques in light of the present disclosure. Appropriate controls take into account the possible cytotoxic effect of a compound, such as treating cells not associated with a cell proliferative disorder (e.g., control cells) with a test compound and can also be used as part of the diagnostic assay.

5 The diagnostic methods of the invention involve the screening for agents that modulate the effects of LP polypeptide-associated disorders.

The LP polypeptides or antibodies thereto as well as LP polypeptide antagonists or agonists can be employed as therapeutic agents. Such therapeutic agents are formulated according to known methods to prepare pharmaceutically useful
10 compositions, whereby the LP polypeptide or antagonist or agonist thereof is combined in a mixture with a pharmaceutically acceptable carrier.

In the case of LP polypeptide antagonistic or agonistic antibodies, if the LP polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver
15 the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by
20 recombinant DNA technology [see, e.g., Marasco, *et al.*, *Proc. Natl. Acad. Sci. USA* 90(16):7889-93 (1993)].

Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers [*Remington's Pharmaceutical Sciences* 16th edition (1980)], in
25 the form of lyophilized formulations or aqueous solutions.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

30 The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate)

microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences, supra*.

5 The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, and intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

10 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent(s), which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels [for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)],
15 polylactides, copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

Microencapsulation of recombinant proteins for sustained release has been successfully
20 performed with human growth hormone (rhGH), interferon, and interleukin-2. Johnson, *et al.*, *Nat. Med.* 2(7):795-9 (1996); Yasuda, *et al.*, *Biomed. Ther.* 27:1221-3 (1993); Hora, *et al.*, *Bio/Technology* 8(8):755-8 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems" in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, Eds.,
25 Plenum Press, NY, 1995, pp. 439-462 WO 97/03692; WO 96/40072; WO 96/07399; and U.S. Patent 5,654,010.

The sustained-release formulations of these proteins may be developed using polylactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids,
30 can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. See Lewis, "Controlled release of bioactive agents from lactide/glycolide

polymer" in *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker; New York, 1990), M. Chasin and R. Langer (Eds.) pp. 1-41.

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 degrees C, resulting in a loss of biological activity and possible changes in immunogenicity.

It is contemplated that the compounds, including, but not limited to, antibodies, small organic and inorganic molecules, peptides, anti-sense molecules, ribozymes, etc., of the present invention may be used to treat various conditions including those characterized by overexpression and/or activation of the disease-associated genes identified herein. The active agents of the present invention (e.g., antibodies, polypeptides, nucleic acids, ribozymes, small organic or inorganic molecules) are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebral, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intraocular, intralesional, oral, topical, inhalation, pulmonary, and/or through sustained release.

Other therapeutic regimens may be combined with the administration of LP polypeptide antagonists or antagonists, anti-cancer agents, e.g., antibodies of the instant invention.

For the prevention or treatment of disease, the appropriate dosage of an active agent, (e.g., an antibody, polypeptide, nucleic acid, ribozyme, or small organic or inorganic molecule) will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the

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determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti and Chappell, "The Use of Interspecies Scaling in Toxicokinetics," in *Toxicokinetics and New Drug Development*, Yacobi, *et al.*, Eds., Pergamon Press, NY, p.4246 (1989).

5 When *in vivo* administration of a composition comprising an LP polypeptide, an LP polypeptide antibody, an LP polypeptide-encoding nucleic acid, ribozyme, or small organic or inorganic molecule is employed, normal dosage amounts may vary from about 1 ng/kg up to 100 mg/kg of mammal body weight or more per day, preferably about 1 pg/kg/day up to 100 mg/kg of mammal body weight or more per day, depending upon
10 the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patents 4,657,760, 5,206,344 or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different
15 from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques
20 and assays.

 In another embodiment of the invention, an article of manufacture containing materials useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers
25 may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is typically an LP polypeptide, antagonist or agonist thereof. The label
30 on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-

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buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

5 LP Polynucleotides and Polypeptides of the Present Invention

The gene encoding human interleukin 25 receptor (IL-25R) has been identified. The encoded IL-25R polypeptide has been characterized as a 502 amino acid protein possessing an N-terminal portion extracellular domain, a single transmembrane region, and a C-terminal portion cytoplasmic tail [Tian *et al.*, *Oncogene* 19:2098-2109 (2000);
10 Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)]. The IL-25R protein is predicted to have a N-terminal signal peptide that either consists of the first 13, 14, 15, or 17 amino acids of the full length protein, depending on the method or program used for prediction of the signal peptide (such as pSORT [Tian *et al.*, *Oncogene* 19:2098-2109 (2000)],
Hidden Markov Modeling [Shi *et al.*, *J. Biol. Chem.* 275:19167-19176 (2001)], SPScan,
15 SigCleave, SignalP, or similar methods). IL-25R contains a putative, single transmembrane region, which is predicted to reside between amino acids 293-309, 288-316, or 290-311 of the full length protein sequence, depending on the analysis method or program used (such as pSORT [Tian *et al.*, *Oncogene* 19:2098-2109 (2000)], TMAP [Milpetz *et al.*, *Trends Biochem Sci.* 20:2-4-205 (1995)], or other similar computer-
20 assisted analyses [Shi *et al.*, *J. Biol. Chem.* 275:19167-19176 (2001)]). Thus, the N-terminal portion of the IL-25R that precedes the transmembrane region is the extracellular portion of the protein. In view of the predicted signal sequence and transmembrane region as described above, the extracellular portion of the mature IL-25R resides within or alternatively is approximately from amino acids 14-292.

25 The gene for human IL-25R has been mapped to 3p21.1 [Tian *et al.*, *Oncogene* 19:2098-2109 (2000), Shi *et al.*, *J. Biol. Chem.* 275:19167-19176 (2001)]. The gene spans a region that is approximately 20 kb in length, and encodes precursor mRNA having 11 exons which are spliced in the processing of the precursor mRNA to yield the mature mRNA which is translated to produce IL-25R polypeptide.

30 Applicants have identified cDNA clones comprising polynucleotides that encode novel polypeptides or novel variants of IL-25 receptor protein:

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1) LP391

LP391 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:1 are contemplated as one embodiment of the present invention. Specifically, polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:2, as well as fragments, variants, and derivatives thereof. Accordingly, LP391 polynucleotides encoding the LP391 polypeptides of the present invention are also contemplated by the present invention.

Sequence analysis of LP391 polypeptide indicates that it is a shortened splice variant of IL-25R, with the LP391 mRNA lacking exon 6 found in IL-25R mRNA. LP391 polypeptide is therefore identical to IL-25R polypeptide with the exception that LP391 lacks amino acids 162-177 of full length IL-25R. The full length LP391 thus possesses a signal peptide and a transmembrane domain (at about amino acids 272-300) corresponding to those found in IL-25R. The mature protein produced following removal of the signal peptide is about 471 amino acids in length (from about amino acid 16 to amino acid 486 of SEQ ID NO:2). Accordingly, the mature, extracellular portion of LP391 is from about amino acid 16 to about amino acid 271 of SEQ ID NO:2.

2) LP392

LP392 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:3 are contemplated as another embodiment of the present invention. LP392 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:4, as well as fragments, variants, and derivatives thereof. LP392 polynucleotides encoding the LP392 polypeptides of the present invention are further contemplated by the present invention.

LP392 polypeptide is a lengthened splice variant of IL-25R, as the LP392 mRNA contains an additional 87 nucleotide exon preceeding exon 9 of IL-25R mRNA. The majority of LP392 is identical to IL-25R, with the exception that LP392 contains an additional 29 extracellular amino acids inserted between amino acids 249 and 250 of full length IL-25R. Full length LP392 polypeptide, like IL-25R, possesses a signal peptide and a single transmembrane domain (at about amino acids 317-345). The mature

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LP392 protein is about 516 amino acids in length (from about amino acid 16 to amino acid 531 of SEQ ID NO:4). Accordingly, the mature, extracellular portion of LP392 is from about amino acid 16 to about amino acid 316 of SEQ ID NO:4.

5 3) LP393

Another embodiment of the present invention is LP393 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:5. LP393 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:6, as well as fragments,
10 variants, and derivatives thereof. Accordingly, LP393 polynucleotides encoding the LP393 polypeptides of the present invention are further contemplated by the present invention.

LP393 polypeptide is a novel splice variant of IL-25R in which the LP393 mRNA is alternatively spliced at the beginning of exon 11 relative to the IL-25R mRNA. The 3'
15 alternative splicing results in the skipping of four bases in the LP393 mRNA compared to that of IL-25R, yielding a frame shift and a corresponding early stop codon in exon 11. Full length LP393 is therefore a 371 amino acid polypeptide in which amino acids 1-315 are the same as amino acids 1-315 in IL-25R. LP393 correspondingly contains a signal peptide and transmembrane domain (at about amino acids 288-316) like those in IL-25R.
20 The mature LP393 protein is about 356 amino acids in length, from about amino acid 16 to amino acid 356 of SEQ ID NO:6. Accordingly, the mature, extracellular portion of LP393 is from about amino acid 16 to about amino acid 287 of SEQ ID NO:6.

4) LP394

25 LP394 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:7 are contemplated as one embodiment of the present invention. Specifically, LP394 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:8, as well as fragments, variants, and derivatives thereof. Accordingly, LP394
30 polynucleotides encoding the LP394 polypeptides of the present invention are also contemplated by the present invention.

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Sequence analysis of LP394 polypeptide indicates that it is a shortened splice variant of IL-25R. In LP394 mRNA, an alternative 3' splice site removes 133 bases of exon 11 relative to the IL-25R mRNA. The loss of these 133 bases yields a frame shift and provides an early stop codon, such that the first 316 amino acids of the full length, 328 amino acid LP394 polypeptide are identical to the corresponding amino acids in IL-25R polypeptide. Accordingly, the full length LP394 has a putative signal peptide (approximately amino acids 1-15) and a putative transmembrane domain (approximately amino acids 288-316) similar to those found in IL-25R. The mature LP394 protein produced following removal of the signal peptide is about 313 amino acids in length (from about amino acid 16 to amino acid 328 of SEQ ID NO:8). Accordingly, the mature, extracellular portion of LP394 is from about amino acid 16 to about amino acid 287 of SEQ ID NO:8.

5) LP395

LP395 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:9 are contemplated as another embodiment of the present invention. LP395 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:10, as well as fragments, variants, and derivatives thereof. LP395 polynucleotides encoding the LP395 polypeptides of the present invention are further contemplated by the present invention.

LP395 polypeptide is a novel splice variant of IL-25R. In comparison to IL-25R mRNA, LP395 mRNA lacks exon 6 and contains an alternative 3' splice site that removes 133 bases of exon 11. The loss of exon 6 in LP395 mRNA yields a LP395 polypeptide that lacks amino acids 162-177 of IL-25R, and the alternative splice site of exon 11 in LP395 mRNA yields a frame shift and provides an early stop codon relative to IL-25R. Accordingly, full length LP395 polypeptide contains 312 amino acids, with amino acids 1-161 and 162-300 of LP395 being identical to IL-25R amino acids 1-161 and 178-316, respectively. Full length LP395, like IL-25R, possesses a signal peptide and a single transmembrane domain (at about amino acids 272-300). The mature LP395 protein is about 297 amino acids in length (from about amino acid 16 to amino acid 312 of SEQ ID

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NO:10). Accordingly, the mature, extracellular portion of LP395 is from about amino acid 16 to about amino acid 271 of SEQ ID NO:10.

6) LP396

5 LP396 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:11 are contemplated as another embodiment of the present invention. LP396 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:12, as well as fragments, variants, and derivatives thereof. LP396 polynucleotides encoding the
10 LP396 polypeptides of the present invention are further contemplated by the present invention.

LP396 polypeptide is a splice variant of IL-25R, wherein LP396 mRNA contains an additional exon preceeding exon 9 of the IL-25R mRNA. This additional exon contains an early stop codon, resulting in a full length LP396 polypeptide of 277 amino
15 acids. The first 249 amino acids of LP396 polypeptide are identical to the first 249 amino acids of IL-25R. LP396 is predicted to be a secreted protein, as it contains a signal peptide (as found in IL-25R) and appears to lack a transmembrane domain. The mature LP396 protein is about 262 amino acids in length (from about amino acid 16 to amino acid 277 of SEQ ID NO:12).

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7) LP397

Another embodiment of the present invention is LP397 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:13. LP397 polypeptides of the present invention
25 comprise the amino acid sequence as shown in SEQ ID NO:14, as well as fragments, variants, and derivatives thereof. Accordingly, LP397 polynucleotides encoding the LP397 polypeptides of the present invention are further contemplated by the present invention.

LP397 polypeptide is a splice variant of IL-25R, with LP397 mRNA lacking
30 exons 8 and 9 of IL-25R mRNA. In addition to lacking codons found in the IL-25R mRNA, the LP397 mRNA contains an early stop codon, thereby yielding a full length LP397 polypeptide that is 252 amino acids in length. Amino acids 1-224 of full length

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LP397 are the same as those found in IL-25R polypeptide. LP397 polypeptide thus contains a signal peptide as found in IL-25R, and is predicted to lack a transmembrane domain. The mature LP397 is predicted to be a secreted protein of about 237 amino acids, from about amino acid 16 to amino acid 252 of SEQ ID NO:14.

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8) LP398

LP398 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:15 are contemplated as another embodiment of the present invention. LP398 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:16, as well as fragments, variants, and derivatives thereof. LP398 polynucleotides encoding the LP398 polypeptides of the present invention are further contemplated by the present invention.

LP398 polypeptide is a splice variant of IL-25R produced from LP398 mRNA in which exon 4 is skipped relative to IL-25R mRNA. The loss of this exon yields a loss of codons and an early stop codon in the LP398 mRNA compared to the IL-25R mRNA. LP398 mRNA thus produces a polypeptide having 96 amino acids, of which the first 75 amino acids are identical to those found in IL-25R. Full length LP398 polypeptide has a signal peptide as found in IL-25R, with the secreted, mature protein being about 81 amino acids in length (from about amino acid 16 to amino acid 96 of SEQ ID NO:16).

9) LP399

Another embodiment of the present invention is LP399 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:17. LP399 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:18, as well as fragments, variants, and derivatives thereof. Accordingly, LP399 polynucleotides encoding the LP399 polypeptides of the present invention are further contemplated by the present invention.

LP399 polypeptide is a splice variant of IL-25R, with LP399 mRNA lacking exons 4, 5, 6, and 7 of IL-25R mRNA. In addition to lacking codons found in the IL-25R mRNA, the LP399 mRNA contains an early stop codon, thereby yielding a full length

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LP399 polypeptide that contains 93 amino acids. Amino acids 1-76 of full length LP399 are the same as those found in IL-25R polypeptide. LP399 polypeptide thus contains a signal peptide as found in IL-25R, and lacks a transmembrane domain. The mature LP399 is predicted to be a secreted protein of about 78 amino acids, from about amino acid 16 to amino acid 93 of SEQ ID NO:18.

10) LP417

LP417 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:19 are contemplated as another embodiment of the present invention. LP417 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:20, as well as fragments, variants, and derivatives thereof. LP417 polynucleotides encoding the LP417 polypeptides of the present invention are further contemplated by the present invention.

LP417 polypeptide is a splice variant of IL-25R. Relative to IL-25R mRNA, exons 3, 4, 5, 6, and 7 are skipped in LP417 mRNA. The loss of these exons yields a loss of codons and an early stop codon in the LP417 mRNA compared to the IL-25R mRNA. LP417 mRNA yields a full length LP417 polypeptide having 46 amino acids, of which the first 28 amino acids are identical to those found in IL-25R. Full length LP398 polypeptide has a signal peptide as found in IL-25R, with the secreted, mature protein being about 31 amino acids in length (from about amino acid 16 to amino acid 46 of SEQ ID NO:20).

11) LP418

Another embodiment of the present invention is LP418 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:21. LP418 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:22, as well as fragments, variants, and derivatives thereof. Accordingly, LP418 polynucleotides encoding the LP418 polypeptides of the present invention are further contemplated by the present invention.

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LP418 polypeptide is a splice variant of IL-25R, with LP418 mRNA lacking exons 5 and 6 of IL-25R mRNA. In addition to lacking codons found in the IL-25R mRNA, the LP418 mRNA contains an early stop codon, thereby yielding a full length LP418 polypeptide that contains 135 amino acids. Amino acids 1-118 of full length LP418 are the same as those found in IL-25R polypeptide. LP418 polypeptide thus contains a signal peptide as found in IL-25R, and lacks a transmembrane domain. The mature LP418 is predicted to be a secreted protein of about 120 amino acids, from about amino acid 16 to amino acid 135 of SEQ ID NO:22.

12) LP419

LP419 polypeptides comprising the amino acid sequence of the open reading frame encoded by the polynucleotide sequence as shown in SEQ ID NO:23 are contemplated as another embodiment of the present invention. LP419 polypeptides of the present invention comprise the amino acid sequence as shown in SEQ ID NO:24, as well as fragments, variants, and derivatives thereof. LP419 polynucleotides encoding the LP419 polypeptides of the present invention are further contemplated by the present invention.

LP419 polypeptide is a splice variant of IL-25R. Compared to IL-25R mRNA, exons 3, 4, and 6 are skipped in LP419 mRNA. The loss of these exons yields a loss of codons and an early stop codon in the LP419 mRNA compared to the IL-25R mRNA. LP419 mRNA yields a full length LP419 polypeptide having 49 amino acids, of which the first 28 amino acids are identical to those found in IL-25R. Full length LP419 polypeptide has a signal peptide as found in IL-25R, with the secreted, mature protein being about 34 amino acids in length (from about amino acid 16 to amino acid 49 of SEQ ID NO:24).

The IL-25 receptor has been termed a member of the IL-17 receptor family, based on its 26% amino acid identity to the IL-17 receptor [Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)]. IL-25R specifically binds IL-25, as binding studies demonstrated that IL-25R strongly bound IL-25, while weak binding of IL-25R was observed with IL-17B, and no binding was detected with either IL-17 or IL-17C [Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)]. IL-25 is structurally related to the IL-17 family of cytokines,

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which includes IL-17, IL-17B, and IL-17C. The IL-17 family members are proinflammatory cytokines whose overlapping biological activities include induction of inflammatory mediators and other cytokines, such as IL-1 β , IL-6, IL-8, and TNF α . Based on studies with human cell lines, IL-25 also has been suggested to be a proinflammatory cytokine, since it was found to induce production of IL-8, and also induced production of NF-kappaB [Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)].

In vivo examinations of IL-25 expression in mice indicate that its biological effects are primarily distinct from those of IL-17, IL-17B, and IL-17C, as IL-25 was found to promote a Th2 immune response [(Fort *et al.*, *Cell* 15:985-995 (2001); Pan *et al.*, *J. Immun.* 167:6559-67 (2001)]. In one study, mice that were treated with purified IL-25 protein experienced induced expression of cytokines IL-4, IL-5, IL-13, eosinophilia, increased serum Ig, and developed significant histological changes in the lungs and the gastrointestinal tract [(Fort *et al.*, *Cell* 15:985-995 (2001)]. In another study, overexpression of IL-25 in mice induced expression of IL-4, IL-10, and IL-13 in several tissues, increased serum IL-5 and IL-13, increased circulating IgE and IgG1, caused neutrophilia and eosinophilia, and caused inflammation in multiple organs, affecting liver, heart, lungs, lymph node, kidneys, spleen, and urinary bladder [Pan *et al.*, *J. Immun.* 167:6559-67 (2001)]. The cytokine production induced by IL-25 and the resultant pathological changes indicate that IL-25 likely is a key cytokine for the development of Th2 immune responses, and accordingly, Th2-associated diseases. Regulation of IL-25 activity will correspondingly prove beneficial for treating several diseases and conditions.

The present invention provides means of regulating IL-25 activity. In particular, soluble LP polypeptides of the present invention or soluble fragments derived from the extracellular portions of LP polypeptides described herein that bind IL-25 may compete with endogenous IL-25 receptor, and thereby antagonize IL-25 activity by sequestering its effects. The ability of LP396, LP397, LP398, LP399, LP417, LP418, and LP419 to antagonize IL-25 will prove useful in the treatment of Th2 associated and other IL-25 mediated diseases. Similarly, the extracellular domains of LP391, LP392, LP393, LP394, and LP395 can be used as therapeutics for Th2 associated diseases. Also, LP391, LP392, and LP395 may bind additional, unidentified ligands which, like IL-25, are involved in Th2 mediated diseases.

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In many diseases, the immune system itself appears to play a significant role in mediating the disease (i.e., the immune system action takes part in actually causing the disease or an inappropriate type of immune response prevents the correct response from irradiating the disease). Many such diseases are thought to involve a pathologic or inappropriate immune response by the humoral branch of the immune system, which is associated with Th2 cell activity. The humoral/Th2 branch of the immune system is generally directed at protecting against extracellular immunogens such as bacteria and parasites through the production of antibodies by B cells; whereas the cellular/Th1 branch is generally directed at intracellular immunogens such as viruses and cancers through the activity of natural killer cells, cytotoxic T lymphocytes and activated macrophages. Th2 cells are believed to produce several cytokines, including IL-3, IL-4, IL-5, IL-10, and IL-13, which are thought to stimulate production of IgE antibodies, as well as be involved with recruitment, proliferation, differentiation, maintenance and survival of eosinophils (i.e., leukocytes that accept an eosin stain), which can result in eosinophilia. Eosinophilia is a hallmark of many Th2 mediated diseases, such as asthma, allergy, and atopic dermatitis.

The LP polypeptides of the present invention and fragments thereof are likely to prove useful in treating asthma through their antagonist effects on IL-25. Consistent with a role of IL-25 in asthma, treatment of mice with IL-25 or overexpression of IL-25 in mice results in histological changes and chronic inflammation in the lungs of these mice [(Fort *et al.*, *Cell* 15:985-995 (2001); Pan *et al.*, *J. Immun.* 167:6559-67 (2001)]. These histological changes were characterized by the presence of eosinophils within the vascular lumen, and infiltrates of eosinophils and/or mononuclear cells beneath the endothelium, within the vessel wall and adjacent to the vessel. In the lung airways, the epithelium lining was hypertrophied and contained high levels of mucus. Chronic pulmonary inflammation involving eosinophil infiltration is a characteristic hallmark feature of bronchial asthma. The role of IL-25 is further demonstrated by the induction of IL-13 by IL-25. In experimental models of asthma, increased mucus production and airway hyperresponsiveness occurs only in the presence of IL-13 [(Fort *et al.*, *Cell* 15:985-995 (2001)]. Mice that were deficient for IL-13 or an IL-13 response did not develop histological changes seen in wild type mice in response to treatment with IL-25 protein [(Fort *et al.*, *Cell* 15:985-995 (2001)]. Antagonism of IL-25 with the LP

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polypeptides described herein will be useful towards treating and preventing histological lung changes induced by IL-25, and thus will be beneficial as a therapeutic for asthma.

In addition to causing inflammation in lungs, increased levels of IL-25 were observed to cause histological changes in a variety of organs and tissues in mice [(Fort *et al.*, *Cell* 15:985-995 (2001); Pan *et al.*, *J. Immun.* 167:6559-67 (2001)]. These tissues include liver, heart, lymph node, kidney, spleen, stomach, small and large intestine, and urinary bladder. Concurrent with these changes, Th2-type inflammatory responses of blood eosinophilia and increased serum levels of IgE and IgG1 were observed. Thus, IL-25 serves as an important mediator of the development of systemic Th2 responses.

The ability of LP polypeptides of the present invention or soluble fragments thereof to antagonize IL-25 will be therapeutically useful in treating a wide range of conditions and diseases that are characterized by a Th2 response. In addition to asthma, diseases that are thought to be caused or mediated in substantial part by Th2 immune response include, without limitation, allergic rhinitis, allergies, dermatitis, systemic lupus erythematosus, Ommen's syndrome (hypereosinophilia syndrome), certain parasitic infections, for example, cutaneous and systemic leishmaniasis, toxoplasma infection and trypanosome infection, and certain fungal infections, for example candidiasis and histoplasmosis, and certain intracellular bacterial infections, such as leprosy and tuberculosis. There is also an association with an increased Th2 response in Hodgkin's and non-Hodgkin's lymphoma as well as embryonal carcinoma.

Moreover, the ability of the LP polypeptides of the present invention to inhibit Th2 response mediated by IL-25 indicate that these LP polypeptides will be useful in treating parasitic infections, for example, cutaneous and systemic leishmaniasis, Toxoplasma infection and Trypanosome infection, certain fungal infections, for example Candidiasis and Histoplasmosis, and intracellular bacterial infections, such as leprosy and tuberculosis. Studies in mice infected with leishmania major have shown that a Th1 response correlates with resistance, whereas a Th2 response correlates with susceptibility. Also studies in mice have shown that parasites that live in macrophages, for example, leishmania major, are killed when the host cells are activated by interferon-gamma, which is known to be a Th1 cell product. In mice infected with candida and histoplasma, it is known that a Th1 response correlates with resistance, whereas a Th2 response correlates with susceptibility.

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Accordingly, LP polypeptides of the present invention or fragments thereof are useful for treating a number of Th2 mediated and other related diseases.

Sequence encoding LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, and LP419 has been localized to human chromosome region

5 3p21.1. This is a region shown to undergo deletions in renal cell carcinoma and chronic myelogenous leukemia [Lee *et al.*, *J. Biol. Chem.* 276:1660-1664 (2001)]. In particular, the following diseases, conditions, syndromes, disorders, or pathological states have also been mapped to this region of the human genome: pancreatic cancer [Shridhar, V. *et al.*, *Oncogene* 14: 2213-2216 (1997)], septooptic dysplasia [Thomas, P. Q. *et al.*, *Hum.*
10 *Molec. Genet.* 10: 39-45 (2001)], glycine encephalopathy [Nanao *et al.*, *Genomics* 19: 27-30 (1994)], mucopolysaccharidosis type IX [Natowicz *et al.*, *New Eng. J. Med.* 335:1029-1033 (1996)], retinopathy (vascular, with cerebral and renal involvement) [Ophoff *et al.*, *Am. J. Hum. Genet.* 69: 447-453 (2001)], Larsen syndrome [Vujic *et al.*, *Am. J. Hum. Genet.* 57:1104-1113 (1995)], spinocerebellar ataxia [Krols *et al.*, *Hum. Genet.* 99:225-
15 232 (1997)], Chanarin-Dorfman syndrome [Lefevre *et al.*, *Am. J. Hum. Genet.* 69:1002-1012 (2001)], ventricular tachycardia, pituitary ACTH-secreting adenoma (both of which are associated with guanine nucleotide-binding protein, alpha-inhibiting activity polypeptide-2) [Magovcevic *et al.*, *Genomics* 12:125-129 (1992); Williamson *et al.*, *Europ. J. Clin. Invest.* 25:128-131 (1995); Lerman *et al.*, *J. Clin. Invest.* 101:2862-2868
20 (1998)], congenital stationary night blindness [Dryja *et al.*, *Nature Genet.* 358-365 (1996)], Hirschsprung disease [Bolk Gabriel *et al.*, *Nature Genet.* 31:89-93 (2002)], long QT syndrome-3 [George *et al.*, *Cytogenet. Cell Genet.* 68:67-70 (1995); Wang *et al.*, *Cell* 80: 805-811 (1995)], progressive type I heartblock and nonprogressive heart block [Schott *et al.* *Nature Genet.* 23:20-21 (1999)], and Brugada syndrome [Chen *et al.*,
25 *Nature* 392:293-295 (1998)]. Accordingly, an isolated and/or recombinant molecule comprising LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419 nucleic acid sequence can be used, for example, to hybridize near a nucleic acid sequence associated with one or more of the above stated diseases, conditions, syndromes, disorders, or pathological states and thus serve as a marker for
30 such a disease gene.

In an embodiment of the invention, therapeutic utility of the LP polypeptide is determined by measuring phosphorylation of tyrosine residues on specific cell lines. The

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early cellular response of cells stimulated with the majority of proteins is protein phosphorylation of the tyrosine residues. This response includes autophosphorylation of corresponding receptors, which thereby leads to the activation of catalytic properties and the initiation of intracellular pathways specific to the cell type. Moreover, signaling downstream of receptors requires phosphorylation of specific kinases inside the cell and other intracellular enzymes of different origin as well as the phosphorylation of multiple adapter/scaffold, structural proteins and transcriptional factors. Therefore, diverse protein-induced cell responses can be visualized by monitoring the state of protein phosphorylation after cell stimulation.

Immunodetection is used to detect the protein phosphorylation of the stimulated cell. Several antibodies that are directed against specific phosphorylated epitopes in signaling molecules are readily available. Two specific antibodies are used: phosphospecific anti-MAPK and anti-AKT antibodies. Additionally, non-specific anti-phosphotyrosine antibodies, which recognize tyrosine-phosphorylated proteins, are used. While anti-phosphotyrosine antibodies allow detection of diverse tyrosine phosphorylated proteins without directly addressing the nature of their identity, the phosphospecific anti-MAPK and anti-AKT antibodies recognize only the corresponding proteins in their phosphorylated form.

Another assay to determine utility of LP polypeptides involves transfection of cell lines with reporter plasmids followed by cell stimulation with an LP polypeptide. Each reporter consists of a defined element, responsive to specific intracellular signaling pathways, upstream of a sequence involving a reporter protein such as luciferase. Upon stimulation of the element, reporter transcription and translation ensues, and the resulting protein levels can be detected using an assay such as a luminescence assay. The cell stimulation period depends on the reporter plasmid used.

For each reporter used, positive controls are designed in the form of agonist cocktails which include approximately maximal stimulatory doses of several ligands known to stimulate the represented signaling pathway. Using this design, the chances of finding a positive stimulus for each cell line is maximized. The caveat, however, is that some cell line / reporter combinations will not be stimulated by the specific agonist cocktail, due to lack of an appropriate ligand in the cocktail. Alternately, the lack of signal induction by an agonist cocktail may be the lack of all signaling components within

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a particular cell line to activate the transcriptional element. Cell line / reporter combinations with no exogenous stimulus added make up the negative controls.

Another assay to determine utility of LP polypeptides involves transfection of cell lines with reporter plasmids followed by cell stimulation with an LP polypeptide. Each reporter consists of a defined element, responsive to specific intracellular signaling pathways, upstream of a sequence involving a reporter protein such as luciferase. Upon stimulation of the element, reporter transcription and translation ensues, and the resulting protein levels can be detected using an assay such as a luminescence assay. The cell stimulation period depends on the reporter plasmid used.

For each reporter used, positive controls are designed in the form of agonist cocktails which include approximately maximal stimulatory doses of several ligands known to stimulate the represented signaling pathway. Using this design, the chances of finding a positive stimulus for each cell line is maximized. The caveat, however, is that some cell line/reporter combinations will not be stimulated by the specific agonist cocktail, due to lack of an appropriate ligand in the cocktail. Alternately, the lack of signal induction by an agonist cocktail may be the lack of all signaling components within a particular cell line to activate the transcriptional element. Cell line / reporter combinations with no exogenous stimulus added make up the negative controls.

In another assay, utility of LP polypeptide is determined by proliferation of cells. In this assay, gross changes in the number of cells remaining in a culture are monitored after exposure to an LP polypeptide for three days. The cells are incubated in an appropriate assay medium to produce a sub-optimal growth rate. For example, usually a 1:10 or 1:20 dilution of normal culture medium results in a 40 to 60% reduction in cell number compared to the undiluted culture medium. This broad growth zone is chosen so that if an LP polypeptide is a stimulator of growth, the cells have room to expand, and conversely, if the LP polypeptide is deleterious, a reduction in cell density can be detected. After a period of exposure, the assay media is replaced with media containing a detection agent such as Calcein AM, a membrane-permeant fluorescent dye, allowing quantification of the cell number.

For use in another assay, a FLAG-HIS (FLIS)-tagged version of the LP polypeptide is expressed using mammalian cells such as HEK-293EBNA, COS-7, or HEK293T. The coding region of the cDNA is amplified by PCR of a vector containing a

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fragment encoding the LP polypeptide. The PCR-generated fragment is cleaved with restriction enzymes and gel-purified. The fragment is then ligated into a mammalian expression vector containing the FLIS epitope tag fused to the C-terminus. Protein expressed by this plasmid construct includes both the FLAG tag (Asp-Tyr-Lys-Asp-Asp-
5 Asp-Asp-Lys) and the 6X His tag at the COOH -terminus of the protein. This tag provides epitopes for commercially available tag-specific antibodies, enabling detection of the protein.

To determine expression of the LP polypeptide in tissues, a protein-binding assay is performed. The fixed tissue sample is exposed to the FLIS-tagged LP polypeptide,
10 followed by exposure to a primary antibody and a secondary antibody containing a fluorescent dye. Tagged LP polypeptide that binds to the antigens in the tissue will fluoresce. Binding of the protein to an antigen in the tissue suggests that the protein is expressed in that tissue. Thus, protein expression can be determined by measuring which tissues fluoresce.

15 Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

20

Example 1

Isolation of IL-25R Splice Variants

Splice variants of the IL-25R gene were amplified by the polymerase chain reaction (PCR). Oligonucleotides that anneal to sequences in the IL-25R gene which correspond to the 5' and 3' untranslated regions (UTRs) of the IL-25R mRNA were
25 designed and PCR was performed. The 25 microliter PCR reaction contained (final concentrations) 1X Hot Start Pfu buffer, 0.2 mM dNTPs, 0.4 μ M of each oligo, 1.25 units of Hot Start Pfu polymerase (Stratagene, La Jolla, CA), and 1 ng of Quickclone human kidney cDNA (Clontech, Palo Alto, CA). The PCR reaction was cycled as follows: step 1, 94° C denaturation for 1 min; step 2, 94° C denaturation for 30 seconds; step 3,
30 annealing at 60° C for 30 seconds; step 4, extension at 72° C for 2 min, step 5, Cycle 29

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more times to step 2; step 6, final extension at 72° C for 5 min, and step 7, 10° C soak forever.

Ten microliters of the PCR reaction were run on a 1% TBE gel at 100 volts for 1 hour to confirm successful amplification. The remaining PCR product was prepared for cloning by adding A overhangs to the PCR reaction. A 10 microliter reaction containing 9.6 microliters of the PCR product, 200 uM dNTPs, and 1 unit of Taq polymerase was incubated at 72° C for 10 minutes.

The PCR product with A overhangs was cloned into the pCRII TOPO vector as described by the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA). Briefly, a 6 microliter ligation reaction was assembled consisting of 3 microliters of the PCR/A overhang product, 0.2 M NaCl / 0.01 M MgCl₂ and 1 microliter of TOPO cloning vector. The ligation reaction was incubated at room temperature for 10 to 30 minutes.

Five microliters of the ligation reaction were used for transformation. DH5alpha competent cells (Invitrogen) were incubated with 5 microliters of the ligation reaction on ice for 30 minutes. The cells were heat shocked at 42° C for 45 seconds and placed on ice for 2 minutes. Nine hundred microliters of SOC medium was added to the transformation mix and the cells were incubated at 37° C for 1 hour with shaking at 220 rpm. Forty microliters of X-gal was spread on top of an LB/ amp plate containing 100 µg/ml of ampicillin for blue/white colony selection. Aliquots of 100 microliters and 50 microliters of cells were plated and allowed to grow overnight for 12 to 18 hours at 37° C.

Ninety-one white colonies were picked from the plate and grown overnight for 16 to 18 hours at 37° C in a 96 well block containing 1.2 ml of Magnificent broth in the presence of 100 µg/ml of ampicillin. The blocks were incubated at 37° C with shaking at 300 rpm. The blocks were centrifuged at 3000 rpm for 10 to 15 minutes at room temperature. The media was decanted and the blocks containing cell pellets were loaded onto the Qiagen BioRobot 8000 (Qiagen, Valencia, CA) for automated plasmid isolation. The isolated plasmid DNA was quantitated on a Spectramax 190 (Molecular Devices Corporation, Sunnyvale, CA). The data was imported into a spreadsheet application where dilution parameters were set for the creation of a diluted DNA plate for DNA sequencing. The dilutions were prepared by a Tecan Genesis robot.

Full-length sequencing was obtained for all 91 clones. The 91 full-length sequences were compared to the wild type, published gene sequence for IL-25R [Lee *et*

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al., *J. Biol. Chem.* 276:1660-1664 (2001); Tian *et al.*, *Oncogene* 19:2098-2109 (2000)]. Clones differing from the published sequence were aligned with genomic sequence to determine if they were splice variants, based on the conserved 5' GT and 3' AG sequences found in introns. The clones determined to be splice variants were further
5 analyzed with the basic local alignment tool (BLAST).

Example 2

Expression and Purification of LP polypeptides in *E. coli*

The bacterial expression vector pQE60 is used for bacterial expression in this
10 example. (QIAGEN, Inc., Chatsworth, CA). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-triacetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., and suitable single restriction enzyme cleavage sites.
15 These elements are arranged such that a DNA fragment encoding a polypeptide can be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, a polypeptide coding sequence can optionally be inserted such that translation of the six His codons is prevented and, therefore, a polypeptide is produced with no 6 X His tag.

20 The nucleic acid sequence encoding the desired portion of an LP polypeptide lacking the hydrophobic leader sequence is amplified from a cDNA clone using PCR oligonucleotide primers (based on the sequences presented, e.g., in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23) which anneal to the amino terminal encoding DNA sequences of the desired portion of the LP polypeptide-encoding nucleic acid and to
25 sequences in the construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning, the 5' and 3' primers have nucleotides corresponding or complementary to a portion of the coding sequence of the LP polypeptide-encoding
30 nucleic acid, e.g., as presented in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, according to known method steps. One of ordinary skill in the art would appreciate; of course, that the point in a polynucleotide sequence where the 5' primer begins can be

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varied to amplify a desired portion of the complete polypeptide-encoding polynucleotide shorter or longer than the polynucleotide which encodes the mature form of the polypeptide.

The amplified nucleic acid fragments and the vector pQE60 are digested with appropriate restriction enzymes, and the digested DNAs are then ligated together. Insertion of the LP polypeptide-encoding DNA into the restricted pQE60 vector places the LP391, LP392, LP393, LP394, LP395, LP396, LP397, LP398, LP399, LP417, LP418, or LP419 polypeptide coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG codon. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook, *et al.*, 1989; Ausubel, 1987-1998. *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing LP polypeptides, is available commercially from QIAGEN, Inc. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing. Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/mL) and kanamycin (25 µg/mL). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-beta-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for three to four hours. Cells then are harvested by centrifugation.

The cells are then stirred for three to four hours at 4 °C in 6 M guanidine hydrochloride, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the LP polypeptide is dialyzed against 50 mM sodium acetate buffer, pH 6, supplemented with 200 mM sodium chloride. Alternatively, a polypeptide can be

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successfully refolded by dialyzing it against 500 mM sodium chloride, 20% glycerol, 25 mM Tris hydrochloride, pH 7.4, containing protease inhibitors.

If insoluble protein is generated, the protein is made soluble according to known method steps. After renaturation, the polypeptide is purified by ion exchange, hydrophobic interaction, and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column is used to obtain pure LP polypeptide. The purified polypeptide is stored at 4 degrees C or frozen at negative 40 degrees C to negative 120 degrees C.

Example 3

Cloning and Expression of LP polypeptides in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding the mature LP polypeptide into a baculovirus, using a baculovirus leader and standard methods as described in Summers, *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 polypeptide and convenient restriction sites such as BamHI, Xba I, and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Other baculovirus vectors are used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow, *et al.*, *Virology* 170:31-39 (1989).

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The cDNA sequence encoding the mature LP polypeptide in a clone, lacking the AUG initiation codon and the naturally associated nucleotide binding site, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or
5 complementary to a portion of the coding sequence of an LP polypeptide-encoding polynucleotide, e.g., as presented in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 27, 19, 21, or 23 according to known method steps.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (e.g., "GeneClean," BIO 101 Inc., La Jolla, CA). The fragment is then
10 digested with the appropriate restriction enzyme and again is purified on a 1% agarose gel. This fragment is designated herein "F1."

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a
15 commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, CA). This vector DNA is designated herein "V1."

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread
20 on culture plates. Bacteria are identified that contain the plasmid bearing a human LP polypeptide-encoding polynucleotide using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing an LP polypeptide-encoding polynucleotide will show amplification of the DNA. The sequence of the cloned fragment is confirmed
25 by DNA sequencing. The resulting plasmid is designated herein as pBacLP.

Five μ g of the plasmid pBacLP plasmid construct is co-transfected with 1.0 μ g of a commercially available linearized baculovirus DNA ("BaculoGold[®] baculovirus DNA", PharMingen, San Diego, CA), using the lipofection method described by Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 7413-7 (1987). 1 μ g of BaculoGold[®]
30 virus DNA and 5 μ g of the plasmid pBacLP are mixed in a sterile well of a microtiter plate containing 50 μ L of serum-free Grace's medium (Life Technologies, Inc., Rockville, MD). Afterwards, 10 μ L Lipofectin plus 90 μ L Grace's medium are added,

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mixed and incubated for fifteen minutes at room temperature. Then, the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 mL Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for five hours at 27 degrees C. After five hours, the transfection solution is removed from the plate and 1 mL of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27 degrees C for four days.

After four days, the supernatant is collected, and a plaque assay is performed.

An agarose gel with "Blue Gal" (Life Technologies, Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies, Inc., Rockville, MD, pp. 9-10). After appropriate incubation, blue stained plaques are picked with a micropipettor tip (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μ L of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later, the supernatants of these culture dishes are harvested and then stored at 4 degrees C.

To verify the expression of the LP polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus at a multiplicity of infection ("MOI") of about two. Six hours later, the medium is removed and replaced with SF900 II medium minus methionine and cysteine (available, e.g., from Life Technologies, Inc., Rockville, MD). If radiolabeled polypeptides are desired, 42 hours later, 5 mCi of 35 S-methionine and 5 mCi 35 S-cysteine (available from Amersham, Piscataway, NJ) are added. The cells are further incubated for sixteen hours and then harvested by centrifugation. The polypeptides in the supernatant as well as the intracellular polypeptides are analyzed by SDS-PAGE, followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified polypeptide can be used to determine the amino terminal sequence of the mature polypeptide and, thus, the cleavage point and length of the secretory signal peptide.

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Example 4

Cloning and Expression of an LP Polypeptide in Mammalian Cells

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the polypeptide coding
5 sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early
10 promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala,
15 Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Other suitable mammalian host cells include human Hela 293, H9, Jurkat cells, mouse NIH3T3, C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells, and Chinese hamster ovary (CHO) cells.

Alternatively, the gene is expressed in stable cell lines that contain the gene
20 integrated into a chromosome. The co-transfection with a selectable marker such as DHFR (dihydrofolate reductase), GPT neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded polypeptide. The DHFR marker is useful to develop cell lines that carry several
25 hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) [Murphy, *et al.*, *Biochem. J.* 227:277-9 (1991); Bebbington, *et al.*, *Bio/Technology* 10:169-75 (1992)]. Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a
30 chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of polypeptides.

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The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus [Cullen, *et al.*, *Mol. Cell. Biol.* 5:438-47 (1985)] plus a fragment of the CMV-enhancer [Boshart, *et al.*, *Cell* 41:521-30 (1985)]. Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI, and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 4(a)

Cloning and Expression in COS Cells

The expression plasmid, pLP HA, is made by cloning a cDNA encoding LP polypeptide into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) or HIS tag (see, e.g., Ausubel, *supra*) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin polypeptide described by Wilson, *et al.*, *Cell* 37:767-8 (1984). The fusion of the HA tag to the target polypeptide allows easy detection and recovery of the recombinant polypeptide with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the LP polypeptide is cloned into the polylinker region of the vector so that recombinant polypeptide expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The LP polypeptide-encoding cDNA of a clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of LP polypeptides in

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E. coli. Non-limiting examples of suitable primers include those based on the coding sequences presented in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23.

The PCR amplified DNA fragment and the vector, pcDNA1/Amp, are digested with suitable restriction enzyme(s) and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, La Jolla, CA), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the LP polypeptide-encoding fragment.

For expression of recombinant LP polypeptide, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook, *et al.*, *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of the LP polypeptide-encoding polynucleotide by the vector.

Expression of the LP polypeptide-HA fusion is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow, *et al.*, *Antibodies: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ³⁵S-cysteine for eight hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM sodium chloride, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson, *et al.*, cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated polypeptides then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 4(b)

Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of the LP polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary cells or other cells lacking dihydrofolate activity that are transfected with

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these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented [see, e.g., Alt, *et al.*, *J. Biol. Chem.* 253:1357-70 (1978); Hamlin and Ma, *Biochem. et Biophys. Acta* 1097:107-43 (1990); and Page and Sydenham, *Biotechnology* 9:64-8 (1991)]. Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus [Cullen, *et al.*, *Mol. Cell. Biol.* 5: 438-47 (1985)] plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) [Boshart, *et al.*, *Cell* 41: 521-30 (1985)]. Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites, the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human beta-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the LP polypeptide in a regulated way in mammalian cells [Gossen, and Bujard, *Proc. Natl. Acad. Sci. USA* 89:5547-51 (1992)]. For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

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The DNA sequence encoding the complete the LP polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequences of an LP polypeptide-encoding polynucleotide, e.g., as presented in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, according to known method steps.

The amplified fragment is digested with suitable endonucleases and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. Five μ g of the expression plasmid pC4 is cotransfected with 0.5 μ g of the plasmid pSV2-neo using lipofectin. The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 μ g/mL G418. After two days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/mL of methotrexate plus 1 μ g/mL G418. After about ten to fourteen days, single clones are trypsinized and then seeded in six-well petri dishes or 10 mL flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new six-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 to 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 5

Tissue Distribution of LP polypeptide-encoding mRNA

Northern blot analysis is carried out to examine expression of LP-polypeptide mRNA in human tissues, using methods described by, among others, Sambrook, *et al.*,

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cited above. A cDNA preferably probe encoding the entire LP polypeptide is labeled with ^{32}P using the RediprimeTM DNA labeling system (Amersham Life Science); according to the manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100TM column (Clontech Laboratories, Inc.) according to the
5 manufacturer's protocol number PT1200-1. The purified and labeled probe is used to examine various human tissues for LP polypeptide mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHybTM hybridization solution (Clontech) according to
10 manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted, exposed to film at negative 70 degrees C overnight, and developed according to standard procedures.

Example 6

Protein Phosphorylation on Tyrosine Residues

15 Protein-induced cell responses are determined by monitoring tyrosine phosphorylation upon stimulation of cells by addition of LP polypeptides. This is accomplished in two steps: cell manipulation and immunodetection.

Protein phosphorylation is measured using the SK-N-MC neuroblastoma cell line
20 (ATCC HTB-10). On day one, the cells are plated into poly-D-lysine-coated, 96 well plates containing cell propagation medium [DMEM:F12 (3:1), 20 mM HEPES at pH 7.5, 5% FBS, and 50 $\mu\text{g}/\text{mL}$ Gentamicin]. The cells are seeded at a concentration of 20,000 cells per well in 100 μL medium. On day two, the propagation medium in each well is replaced with 100 μL starvation medium containing DMEM:F12 (3:1), 20 mM HEPES at
25 pH 7.5, 0.5% FBS, and 50 $\mu\text{g}/\text{mL}$ Gentamicin. The cells are incubated overnight.

On day three, pervanadate solution is made ten minutes before cell lysis; pervanadate is prepared by mixing 100 μL of sodium orthovanadate (100 mM) and 3.4 μL of hydrogen peroxide (producing 100X stock pervanadate solution). The lysis buffer is then prepared: 50 mM HEPES at pH 7.5, 150 mM sodium chloride, 10%
30 glycerol, 1% TRITON-X100TM, 1 mM EDTA, 1 mM pervanadate, and BM protease inhibitors. The cells are stimulated by adding 10 μL of the LP polypeptide solution to the cells, and incubating for ten minutes. Next, the medium is aspirated, and 75 μL lysis

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buffer are added to each well. The cells are lysed at 4 degrees C for fifteen minutes, then 25 μ L of 4X loading buffer are added to the cell lysates. The resultant solution is mixed then heated to 95 degrees C.

Detection of tyrosine phosphorylation is accomplished by Western
5 immunoblotting. Twenty microliters of each cell sample are loaded onto SDS-PAGE eight to sixteen percent amino acid-ready gels from Bio-Rad, and the gels are run. The proteins are electrotransferred in transfer buffer (25 mM Tris base at pH 8.3, 0.2 M glycine, 20% methanol) from the gel to a nitrocellulose membrane using 250 mA per gel over a one hour period. The membrane is incubated for one hour at ambient conditions
10 in blocking buffer consisting of TBST (20 mM Tris hydrochloride at pH 7.5, 150 mM sodium chloride, 0.1% TWEEN[®]-20) with 1% BSA.

Next, the antibodies are added to the membrane. The membrane is incubated overnight at 4 degrees C with gentle rocking in primary antibody solution consisting of the antibody, TBST, and 1% BSA. The next day, the membrane is washed three times,
15 five minutes per wash, with TBST. The membrane is then incubated in the secondary antibody solution consisting of the antibody, TBST, and 1% BSA for one hour at ambient conditions with gentle rocking. After the incubation, the membrane is washed four times with TBST, ten minutes per wash.

Detection is accomplished by incubating the membrane with 10 to 30 mL of
20 SuperSignal Solution for one minute at ambient conditions. After one minute, excess developing solution is removed, and the membrane is wrapped in plastic wrap. The membrane is exposed to X-ray film for twenty second, one minute, and two minute exposures (or longer if needed). The number and intensity of immunostained protein bands are compared to bands for the negative control-stimulated cells (basal level of
25 phosphorylation) by visual comparison.

Example 7

Cell Stimulation with Detection Utilizing Reporters

Protein-induced cell responses are measured using reporters. The SK-N-MC cell
30 line (neuroblastoma; ATCC HTB-10) / NF κ B reporter combination is used.

For the reporter used, positive controls are designed in the form of agonist cocktails. These cocktails include approximate maximal stimulatory doses of several

ligands known to stimulate the regulated signal pathway. For the NFκB reporter, the NFκB / PkC pathway is stimulated by an agonist cocktail containing LPS and TNF-alpha as positive controls. Cell lines and reporters with no exogenous stimulus added are used as negative controls.

At time zero, the cells are transiently transfected with the reporter plasmids in tissue culture flasks using a standard optimized protocol for all cell lines (see Example 1). After 24 hours, the cells are trypsinized and seeded into 96-well poly-D-lysine coated assay plates at a rate of 20,000 cells per well in growth medium. After four to five hours, the medium is replaced with serum-free growth medium. At that time, stimulants for those reporters which required a 24-hour stimulation period are added. After 48 hours, stimulants for the reporters which required a five-hour stimulation period are added. Five hours later, all conditions are lysed using a lysis/luciferin cocktail, and the fluorescence of the samples is determined using a Micro Beta reader.

Each assay plate is plated to contain four positive control wells, sixteen negative control wells, and sixty-four test sample wells (two replicates of thirty-two test samples). The threshold value for a positive "hit" is a fluorescence signal equal to the mean plus two standard deviations of the negative control wells. Any test sample that, in both replicates, generates a signal above that threshold is defined as a "hit."

20 Example 8

Cell Proliferation and Cytotoxicity Determination Utilizing Fluorescence Detection

This assay is designed to monitor gross changes in the number of cells remaining in culture after exposure to LP polypeptides for a period of three days. The following cells are used in this assay:

25 U373MG (astrocytoma cell line, ATCC HTB-22)
T1165 (plastocytoma cell line)
ECV304 endothelial cell line)

30 Prior to assay, cells are incubated in an appropriate assay medium to produce a sub-optimal growth rate, e.g., a 1:10 or 1:20 dilution of normal culture medium. Cells are grown in T-150 flasks, then harvested by trypsin digestion and replated at 40 to 50% confluence into poly-D-lysine-treated 96-well plates. Cells are only plated into the inner

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thirty-two wells to prevent edge artifacts due to medium evaporation; the outer wells are filled with buffer alone. Following incubation overnight to stabilize cell recovery, LP polypeptides are added to the appropriate wells. Each polypeptide is assayed in triplicate at two different concentrations, 1X and 0.1X dilution in assay medium. Two controls are also included on each assay plate: assay medium and normal growth medium.

After approximately 72 hours of exposure, the plates are processed to determine the number of viable cells. Plates are spun to increase the attachment of cells to the plate. The medium is then discarded, and 50 μ L of detection buffer is added to each well. The detection buffer consisted on MEM medium containing no phenol red (Gibco) with calcein AM (Molecular Probes) and PLURONIC[®] F-127 (Molecular Probes), each at a 1:2000 dilution. After incubating the plates in the dark at room temperature for thirty minutes, the fluorescence intensity of each well is measured using a Cytofluor 4000-plate reader (PerSeptive Biosystems). For a given cell type, the larger the fluorescence intensity, the greater the number of cells in the well. To determine the effects on cell growth from each plate, the intensity of each well containing cells stimulated with an LP polypeptide is subtracted from the intensity of the wells containing assay medium only (controls). Thus, a positive number indicated stimulation of cell growth; a negative number indicated a reduction in growth. Additionally, confidence limits at 95 and 90% are calculated from the mean results. Results lying outside the 95% confidence limit are scored as "definite hits." Results lying between the 95 and 90% confidence limits are scored as "maybes." The distinction between definite hits and maybes varied due to intraplate variability; thus, subjective scoring is used as a final determination for "hits."